Vol. XVII DECEMBER, 1931 No. 3

The Journal of Laboratory and Clinical Medicine

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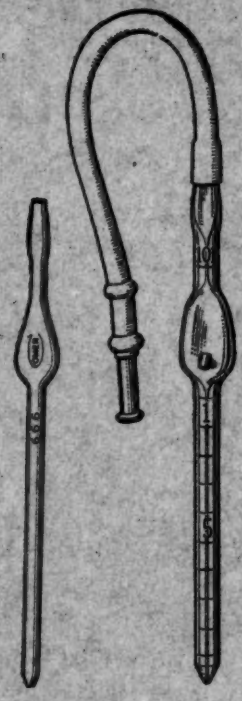

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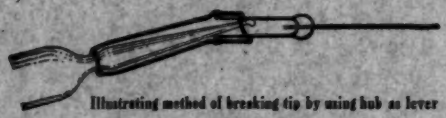

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The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., DECEMBER, 1931 No. 3

CLINICAL AND EXPERIMENTAL

THE DECOMPENSATED HYPERTENSIVE HEART*

BY JAMES R. LISA, M.D., NEW YORK, N. Y.

DURING the course of studies on hypertension, certain changes in the heart were found which seem to have some relationship to the decompensation which sometimes occurs.

This report is based on ten cases. They all had one feature in common, congestive type of cardiac failure with dyspnea on exertion, cyanosis and edema. The blood pressures ranged from 160 to 255.

The hypertrophy was marked in all, the weights ranging from 450 gm. to 1110 gm. With the exception of the presence of many small scars the myocardium presented no marked gross changes. Infarction was absent in all. Its consistence was more or less diminished and its color slightly mottled yellow-brown-red. Microscopically very numerous necrotic foci were found, most frequent in the left ventricle and the interventricular wall though present to a much lesser degree in the other chambers.

The youngest lesion involves only an individual or a very few fibers and consists of a granular degeneration of the cytoplasm, sometimes with, many times without, a cellular infiltration. When present it is usually polynuclear but rapidly becomes mixed cell in type. The cytoplasm rapidly disintegrates and apparently liquefies, when the nuclear remains and the infiltration of cells are more prominent. The edges of the adjacent fibers have cross striations consisting of a row of coarse granules. The lesion sometimes seems to be of more rapid evolution and leaves only a network of capillaries with disintegrating muscle fibers and very slight cellular reaction. Occasionally the cell reaction is of a sharply polynuclear character. More frequently it consists

*From the Pathological Laboratory, City Hospital, Department of Hospitals, Welfare Island.

Received for publication, July 3, 1931.

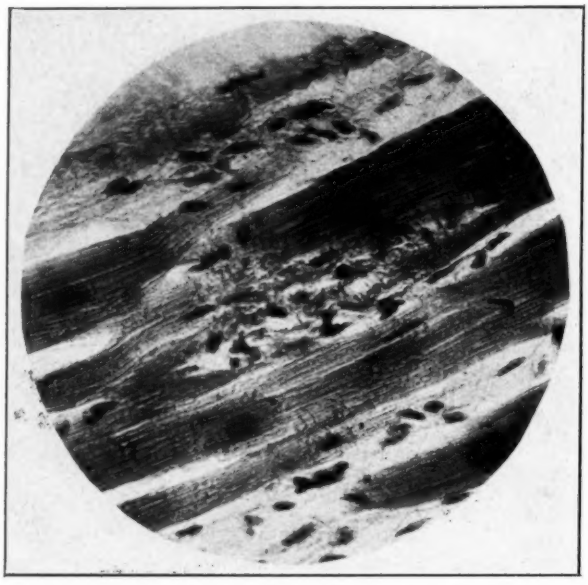


Fig. 1.—Early acute miliary necrosis showing granular degeneration and slight infiltration. In the upper portion of the field is the edge of neighboring lesion showing the fibrillary structure and early connective tissue reaction.

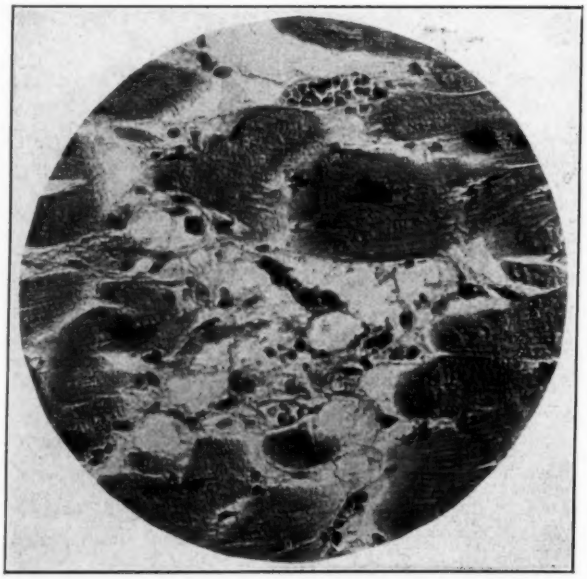


Fig. 2.—A slightly more advanced lesion with almost complete disappearance of the cytoplasm and cellular reaction of lymphocytes, monocytes and a very few polynuclears. The neighboring fibers have the earlier granular appearance and loss of striations.

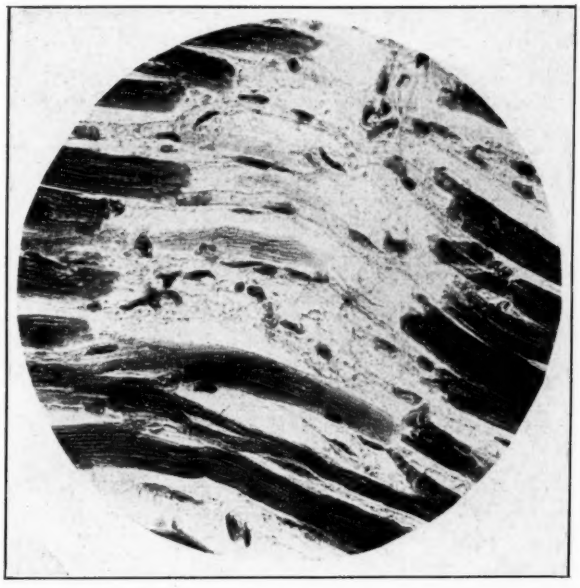


Fig. 3.—A more fulminant type of lesion with extremely acute degeneration of the muscle fibers leaving the capillary network and with very mild infiltration along one edge.

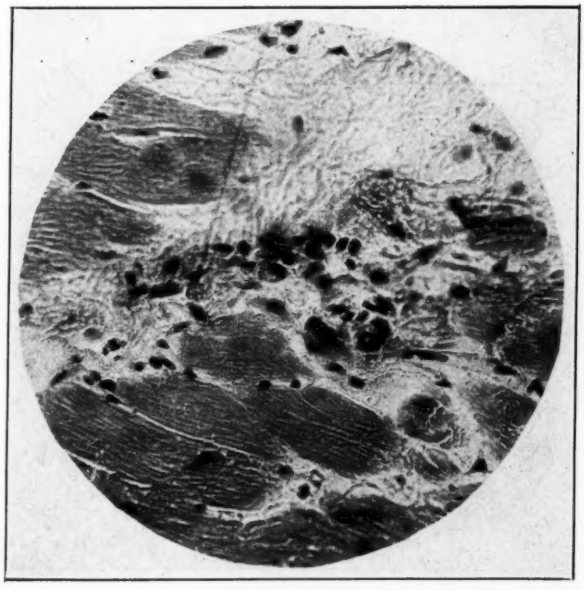


Fig. 4.—A slightly later stage than Fig. 2, with a marked cellular reaction, chiefly monocytic.

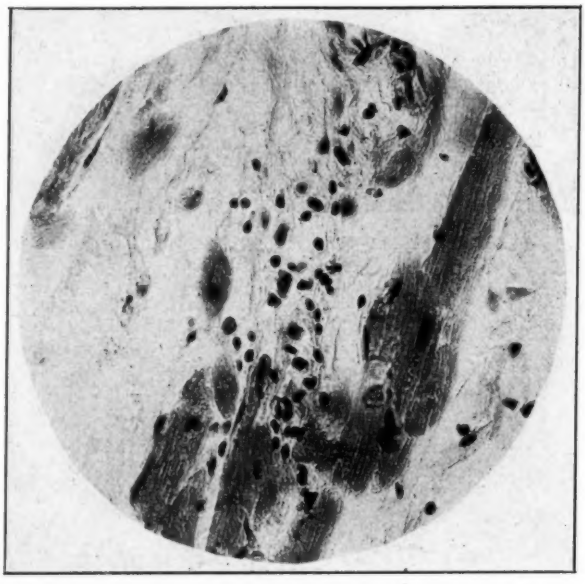


Fig. 5.—A later phase with the predominant infiltration lymphocytic in character and with early connective tissue reaction.

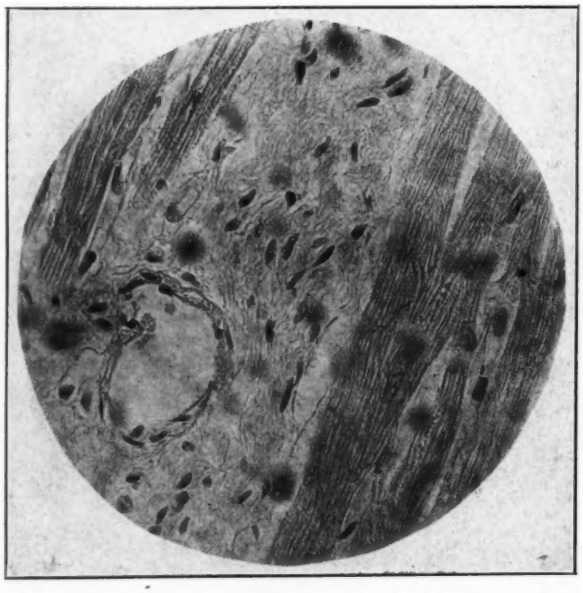


Fig. 6.—A miliary scar with young connective tissue and almost complete disappearance of lymphocytes.

of polynuclears, monocytes and lymphocytes in varying proportion. As the connective tissue begins to proliferate, the lymphocytes tend to predominate and finally completely disappear, leaving only a miliary scar.

Associated with these necroses the myocardium presents a marked fragmentation and areas of fine wavy fibrillae. These resemble very closely some of the lesions found in isolated acute myocarditis.

Three of the cases were associated with syphilis. Spirochetes however could not be demonstrated in any of the hearts from these cases. Syphilis was most likely simply an associated disease unrelated to the hypertension. All the cases were examined for the presence of bacteria with negative results.

CONCLUSIONS

1. Microscopic lesions of the heart in a series of ten cases of hypertension with congestive cardiac failure are presented.

2. They consist of miliary necroses with cellular reaction.

3. The histogenesis is traced.

4. They apparently have a close relationship to the clinical syndrome and offer a pathologic basis for it.

Thanks are due to Miss L. B. Miller for the microphotographs.

RELATION OF JAUNDICE TO THE OUTCOME IN LOBAR PNEUMONIA: INDICATIONS FOR THE TRIAL OF BILIRUBIN THERAPY*

By Norman W. Elton, M.D., D.N.B., Boston, Mass.

IN SEPTEMBER, 1928, a study was undertaken to determine whether or not jaundice was constantly associated with lobar pneumonia,[9] and if so, to analyze its changes in character and intensity in relation to the progress of the disease during its acute course. Such a study was considered advisable because of the possibilities suggested by the presence of jaundice in a disease caused by a bile-soluble organism.[23] The method consisted of the daily determination of three tests with blood serum; the icterus index,[4] the aqueous (direct) van den Bergh reaction,[3] and the quantitative serum bilirubin estimation.[31] The study was based entirely on qualitative and quantitative findings in terms of these three tests, and the trend of the icterus, shown in the daily changes in each test, was correlated as closely as possible with the events of the disease. Hence, it may be described as a study of icterus kinetics. At present 224 cases have been observed in this manner from four different hospitals (Highland Park General Hospital, Michigan; Detroit Receiving Hospital, Michigan; St. Mary's Hospital, Detroit, Michigan; and the Boston City Hospital, Massachusetts). Icterus, manifested in one or all of the three tests, was found to be practically universally present in lobar pneumonia, although it was often encountered in a receding phase when the patient was first seen.

The work had not progressed far before it became evident that it was not a simple matter, but had many different aspects which required detailed consideration. No short space of time was consumed in becoming familiar with the technic and interpretation of the three tests, and it was found necessary, in order to understand the icterus of lobar pneumonia, to undertake an extensive study of jaundice as it runs its course in other clinical entities. The results of this extension of the scope of the investigation are published separately.[9]

During the study of the first series of pneumonias many confusing factors were involved, particularly the outbreak of an influenza epidemic in 1928-1929, during which many cases were quite atypical. Confusion arose from an attempt to classify cases on the basis of the temperature charts, and it was later found that the key to proper classification was the recognition of another factor related conjointly with icterus to the outcome, fluid pleural exudate. The study of these exudates became fully as important as the study of the icterus itself, for when they were present not only did the icterus tend to recede, but the mortality rate was greatly reduced.

*From the Department of Pathology, Detroit College of Medicine and Surgery, and the Fifth Medical (Boston University Teaching) Service, Boston City Hospital.
Received for publication, July 15, 1931.

To present the findings as clearly as possible it has been necessary to classify cases on the basis of the presence or absence of pleural fluid in relation to the maximum icterus index attained during the acute course. Until the recent series at the Boston City Hospital were studied by routine chest-tapping, many cases could not be placed in either of these groups because of incomplete data, although in most of such doubtful cases recovery with effusion was indicated by the dropping of the icterus index to normal during the acute course. It was evident that whenever pleural fluid was diagnosed by x-ray, chest-tap, or careful physical examination, the drop of the icterus index signaled its presence either as or shortly after the fluid developed, but it remained to be demonstrated that whenever this drop occurred, fluid should be expected. For this reason it was essential to obtain conclusive data by routine chest-tapping in an adequate number of well studied cases.

1. *Highland Park General Hospital ("Orientation Series") Table I.*—This series comprises 70 cases in white adults studied during the year 1928-1929. Influenzal cases are included, accounting for many of the fatalities in the normal zone of the icterus index. The clinical data on these cases have already been published.[9] They are here reclassified on the basis of the presence or absence of pleural fluid. Certain observations applicable to the white race may be made from a study of this chart which are consistently upheld by those that follow: (1) In the absence of pleural fluid the mortality is 100 per cent unless the icterus index exceeds 16.6. (2) In the icterus index zone 18.7-30 there is no mortality with or without fluid. (3) In the presence of fluid patients entering the 11-15 zone of the icterus index invariably recover.

TABLE I

HIGHLAND PARK GENERAL HOSPITAL
ORIENTATION SERIES
WHITE RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID		FLUID PRESENT		SUSPECTED FLUID		TOTAL DEATHS
		NO. CASES	DEATHS	NO. CASES	DEATHS	NO. CASES	DEATHS	
-6	27	1	1	17	4	9	3	8
7-10	19	8	8	5	1	6	1	10
11-15	15	4	4	5	0	6	0	4
16.6	3	3	3	0	0	0	0	3
18.7-30	6	4	0	2	0	0	0	0
Totals	70	20	16	29	5	21	4	25

2. *Detroit Receiving Hospital. White Race. (Including Cases Observed at St. Mary's Hospital, Detroit, Michigan.) Table II.*—This series comprises 74 white adults studied during the year 1929-1930. Positive diagnosis of fluid was made in 12 by x-ray, in 6 by physical examination, in 9 by chest-tap, and in 1 by autopsy. There were 10 autopsies in cases when death occurred without fluid. The greater number were typed and many had serial blood cultures. In two cases the icterus index exceeded 30, one patient dying with a type II septicemia at 37.5, the other recovering by true crisis at 100. Several of these cases were obtained only by autopsy and the icterus

determined in the postmortem blood, which partially accounts for the relative increase in the number of deaths with dry pleural cavities. It was impossible to study many patients who may have recovered with fluid, since only those in Detroit Receiving Hospital were closely observed.

TABLE II

DETROIT RECEIVING HOSPITAL SERIES
WHITE RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID			FLUID PRESENT			SUSPECTED FLUID			TOTAL DEATHS
		NO.	CASES	DEATHS	NO.	CASES	DEATHS	NO.	CASES	DEATHS	
-6	11		0	0	8		1	3		0	1
7-10	30		9	9	12		4	9		0	13
11-15	17		9	9	7		0	1		0	9
16.6	5		5	5	0		0	0		0	5
18.7-30	9		7	0	1		0	1		0	0
37.5	1		1	1	0		0	0		0	1
100	1		1	0	0		0	0		0	0
Totals	74		32	24	28		5	14		0	29

3. *Detroit Receiving Hospital. Black Race. Table III.*—From a study of but 46 cases in negroes, it is at once evident that the relationship of icterus to the outcome is strikingly different in this race. Fluid was diagnosed in 15 cases by x-ray, in 6 by physical examination, in 4 by chest-tap, and in 1 by autopsy. There were 6 autopsies in patients dying without fluid. From the data of this table the following observations may be made: (1) The icterus index 16.6 bears no significance. (2) In the absence of pleural fluid the mortality is 100 per cent unless the icterus index exceeds 100. (3) In the presence of fluid, although the mortality rate is greatly reduced, no zone of assured recovery can reasonably be indicated in only a single series. The similarity between the curves of the icterus index in negroes with those of patients apparently white who exceed 30, is striking, for in the absence of fluid the end point for recovery appears to be the same (icterus index 100).

TABLE III

DETROIT RECEIVING HOSPITAL SERIES
BLACK RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID			FLUID PRESENT			SUSPECTED FLUID			TOTAL DEATHS
		NO.	CASES	DEATHS	NO.	CASES	DEATHS	NO.	CASES	DEATHS	
-6	12		1	1	8		3	3		0	4
7-10	9		1	1	7		2	1		0	3
11-16.6	11		2	2	7		0	2		0	2
18.7-30	6		3	3	2		0	1		0	3
37.5	2		2	2	0		0	0		0	2
50	1		1	1	0		0	0		0	1
75	1		0	0	1		1	0		0	1
84	1		1	1	0		0	0		0	1
85.7	1		0	0	1		0	0		0	0
120	1		1	0	0		0	0		0	0
222	1		1	0	0		0	0		0	0
Totals	46		13	11	26		6	7		0	17

TABLE IV

BOSTON CITY HOSPITAL SERIES
FIFTH MEDICAL SERVICE
WHITE RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID		FLUID PRESENT		TOTAL DEATHS
		NO. CASES	DEATHS	NO. CASES	DEATHS	
-6	7	1	1	6	0	1
7-10	11	3	3	8	2	5
11-15	8	2	2	6	0	2
16.6	2	1	1	1	1	2
18.7-30	4	2	0	2	0	0
43	1	1	1	0	0	1
100	1	1	0	0	0	0
Totals	34	11	8	23	3	11

TABLE V

CLINICAL SUMMARY

SERIES AND YEAR	INCIDENCE OF EFFUSION		RELATION TO MORTALITY		
	PROVED	PROVED AND SUSPECTED	MORTALITY WITHOUT FLUID	MORTALITY WITH FLUID	
				PROVED	PROVED AND SUSPECTED
Highland Park General Hospital, Michigan, 1928-1929	41.4%	71.5%	80.0%	17.2%	18.0%
Detroit Receiving Hospital, Michigan, 1929-1930	37.8%	56.8%	75.0%	17.8%	11.9%
Detroit Receiving Hospital, Black Race, 1929-1930	56.5%	72.0%	84.6%	23.0%	18.2%
Boston City Hospital, Massachusetts, 1930-1931	67.6%	—	72.7%	13.0%	—
Total Series, Percentages for White Race	45.0%	64.6%	76.2%	16.3%	14.8%

4. *Boston City Hospital, Fifth Medical Service. White Race. Table IV.*— This group of 34 cases studied during the year 1930-1931 is presented in detail on the chart of the clinical protocol as well as on the general classification table. There were none in which doubt existed as to the presence or absence of fluid. From a study of the findings in the white race the following observations can be made: (1) In the absence of fluid the mortality is 100 per cent in the icterus index zones "normal" to 16.6, and above 30 to an undetermined point below 100. There were no deaths in the zone 18.7-30, or, when the icterus index exceeded 30, if it attained 100. (2) In the presence of pleural fluid there was no mortality if the icterus index exceeded 10 but did not attain 16.6. (3) Pneumococcus septicemia tended to subside spontaneously in the presence of effusion (noted also in the Receiving Hospital series when no specific antibodies were given). (4) Pneumococcus septicemia was often demonstrable with icterus indices at or below 16.6, and occasionally above 30, but has not yet been encountered in cases entering and not exceeding the zone 18.7-30, except in the negro race. (5) Prognoses could not be made from the visible manifestations of icterus, but icterus could be seen in a large number of cases if the sclerae were carefully examined daily. (6) Profuse perspiration frequently accompanied the development of fluid. (7)

TABLE VI

CASE PROTOCOL BOSTON CITY HOSPITAL SERIES 1930-1931

SERIES NO.	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGNS OF FLUID	CHEST TAPS						KINETICS OF SERUM ICTERUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA
				DAY OF TAP	DESCRIPTION OF FLUID	ICTERUS IN FLUID: ICTERUS INDEX	ICTERUS IN FLUID: DIRECT REACTION	ICTERUS IN FLUID: BILIRUBIN	FLUID CULTURE			
1	♂ 42	XVIII	None	4	Dry					4th day Index: 6 Direct reaction: del. 4 min. Bilirubin: 0.5	Died 4th day. Under influence of veronal (addict). Entire left lung consolidated. Temp. 104: pulse 120-160: resp. 60-80. No sputum	Type 18 obtained by lung puncture. W.B.C. 24,000; polys 91%. Blood culture negative 3d day
2	♂ 38	I	None							5th and 7th days Index: 5, 5 Direct reaction: negative Bilirubin: Below 0.2	Consolidation R. middle lobe on 4th day (x-ray) interlobar fluid on 9th day (fluoroscopy) T.P.R. septic to 10th day	Blood culture negative 6th day. W.B.C. 22,000, polys 80% 5th day
3	♂ 36	?	2d to 6th day	4	100 c.c. Amber Turbid	10	Neg.	0.6	N. G.	4th day Index: 5 Direct reaction: negative Bilirubin: Below 0.2	Chill, rusty sputum for 24 hr., while on ward recovering from acute rheumatic fever. Right base. Temp. fell to normal from 103 following tap.	Cell studies typical of pneumonic effusion
4	♀ 21	VIII	6th to 12th day							9th day Index: 5 Direct reaction: positive Bilirubin: 0.33	Rusty sputum first 4 days. Private patient of Dr. John A. Foley	Type 8 from sputum
5	♂ 47	III	Constant	14	60 c.c. Amber Turbid				N. G.	From 13th day Index: 6, 6 Direct reaction: del. 4, neg. Bilirubin: 0.32, 0.25	Admitted because of persistent dyspnea following chill 11 days before. No rusty sputum. Herpes on lips. No history or signs of tuberculosis. Right chest. T.P.R. septic throughout. Released to another hospital	Type III from sputum. Sputum consistently negative for tubercle bacilli. Guinea pig inoculation with fluid negative. Cell studies typical of pneumonic effusion with no evidence of empyema. W.B.C. 20,500, 21,980, 19,900, 14,550 polys 90%, 80%, 80%, 67%
				16	60 c.c. Amber Turbid				N. G.			
				19	60 c.c. Amber Turbid				N. G.			
				27	60 c.c. Yellow Clear				N. G.			
				39	60 c.c. Yellow Clear	3	Neg.	0.22	Guinea pig neg.			
6	♂ 18	I	3d to 5th day	4	10 c.c. Green Turbid	5	Neg.	0.68	N. G.	4th day Index: 6 Direct reaction: neg. Bilirubin: 0.42	Temp. fell by "crisis" on 5th day from 103 before serum could be given. X-ray negative on 4th day.	Blood cultures negative 3d and 4th days W.B.C. 26,000; polys 95%
				5	10 c.c. Yellow Turbid				N. G.			
7	♂ 21	I	6th to 10th day	6	8 c.c. Yellow Turbid				N. G.	From 6th day Index: 5, 3.8, 4 Direct reaction: del. 8, del. 10, neg. Bilirubin: 0.3, 0.26, 0.2	Treated with Felton's specific antibodies 6th and 7th days. Temp. fell by "crisis" on 8th day	Blood cultures negative 6th to 8th days W.B.C. 23,000; polys 92%
				8	2 c.c. Yellow Clear				N. G.			

TABLE VI—CONT'D

8	♀ 63	?	None							Day of death Index: 10 Direct reaction: positive Bilirubin: 0.5	Died 4 hr. after admission on 6th day. R. lower lobe	Autopsy—Gray consolidation right lower lobe. Fibrinous exudate. Dry pleural cavity
9	♀ 42	III	None							Day of death Index: 10 Direct reaction: positive Bilirubin: 0.7	Right upper and middle lobes. Died 6th day	Postmortem taps—no fluid in either pleural cavity
10	♂ 70	II	None							Day of death Index: 10 Direct reaction: positive Bilirubin: 0.4	Right upper lobe. Died 5th day. Temp. 100-101: 103 terminal. Pulse 100-120. Resp. 30-45	Blood culture positive. Autopsy—gray consolidation right upper lobe
11	♂ 38	I	3d to 5th day	3 4	15 c.c. Amber Turbid Dry	6	Neg.	0.34	Type I	From 3d day Index: 6, 6, 10 Direct reaction: positive throughout Bilirubin: 0.35, 0.39, 0.62	Died 6th day. Serum therapy 4th day. Rales of resolution 5th day. Chill 12 hr. before death, followed by delirium. Skin jaundice 5th day	Autopsy—resolving pneumonia left lower lobe, dense fibrin and 150 c.c. fluid in left axillary line. Very recent consolidation at right apex. Blood culture 674 col. per c.c. 3d day, 337 col. before serum. Still positive, but no colonies, to death.
12	♂ 42	III	8th to 9th day	8	10 c.c. Yellow Turbid	3	Pos.	0.32	Type III	From 8th day Index: 7.5, 5 Direct reaction: positive, positive Bilirubin: 0.25, 0.22	Died 9th day. Influenzal onset (mild epidemic prevalent) his 4th attack. Temp. 100 at death. Pulse 120-140. Right lower and middle lobes.	Blood cultures positive 8th-9th days
13	♀ 28	I	6th to 10th day							2d, 4th, 7th, 9th, 12th days Index: 7.5, 6, 6, 6, 6 Direct reaction: neg., pos., pos., pos., neg. Bilirubin: 0.72. 0.26, 0.26, 0.26, 0.26	Rusty sputum first day only. R. middle and lower lobes (x-ray). Signs indefinite until those of fluid on 6th day. Nonspecific serum given 3d day; specific antibodies given 5th day. Parotitis. Respirations 25 to 30 day. Lysis.	Blood cultures positive before and negative after both nonspecific and specific serum W.B.C. 26,000, 60,000, 22,000.
14	♂ 16	II	8th to 16th day							From 4th day Index: 9.3, 5 Direct reaction: del. 5, neg. Bilirubin: 0.6, 0.25	Treated with specific antibodies (Felton's) on 5th and 6th days. Temp. fell from 103 to 95 on 6th day, returning for septic course (100-100.5) for few days. Fluid at left base (x-ray)	Blood culture negative

TABLE VI—CONT'D

SERIES NO.	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGNS OF FLUID	CHEST TAPS						KINETICS OF SERUM ICTERUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA
				DAY OF TAP	DESCRIPTION OF FLUID	ICTERUS IN FLUID: ICTERUS INDEX	ICTERUS IN FLUID: DIRECT REACTION	ICTERUS IN FLUID: BILIRUBIN	FLUID CULTURE			
15	♂ 22	Group IV	6th to 13th day	9	30 c.c. Brown Turbid	6	Del. 1 Min.	0.48	N. G.	From 6th day Index: 6 10, 6, 5, 4.3, 3.5, 3.5 Direct reac.: del. 4. pos. to 11th day, neg. Bilirubin: 0.4, 0.5, 0.56, 0.44, 0.2, 0.2 ∠.2	Treated with types I and II antibodies on 6th day. Lysis terminated 10th day. Right lower lobe.	
16	♀ 43	XIV	9th to 15th day							From 9th day Index: 6.4, 7.5, 6 Direct reac.: pos., pos., neg. Bilirubin: 0.46, 0.41, 0.25	Left lower lobe. Temp. down by lysis 10th day.	W.B.C. 12,800 on 12th day
17	♂ 19	I	9th to 11th day	9	1 c.c. Yellow Turbid				No organisms	From 2d day Index: 6, 5, 7.5, 10, 4.3, 11th day 4.3 Direct reac.: del. 2, pos. to 6th day, neg. Bilirubin: 0.36, 0.38 0.5, 0.2. 11th day ∠.2	Influenzal onset. 2d attack in 6 mo. Specific antibody therapy. Right upper lobe. Fluid obtained from base.	Blood cultures negative 2d to 5th days. W.B.C. 26,300; polys 87%. Kahn and Wassermann positive.
18	♂ 27	I	6th to 12th day	5	0.5 c.c. Amber Turbid				N. G.	From 4th day Index: 7.5, 7.5, 5.3, 4.3, 5, 6, 3 Direct reaction: del. 6. del. 1½, del. 2½, pos., pos., del. 5, neg. Bilirubin: 0.5, 0.2, 0.2, 0.33, 0.23, ∠.2, ∠2	Specific antibody therapy 5th day. "Crisis" on 6th day. Delirium tremens. Right lower lobe.	Blood cultures negative 4th and 5th days W.B.C. 22 000; polys 93% 4th day W.B.C. 11,600; polys 84% 9th day
				9	20 c.c. Amber Turbid				N. G.			
19	♂ 45	I	None							From 4th day Index: 13.6, 11.6, 15, 11.6, 10, 15 Direct reaction: positive throughout Bilirubin: 0.91, 0.6, 1.0, 0.62, 0.3, 0.5	Died 9th day. Nonspecific (antimeningococcic) serum therapy 4th day. Right middle lobe only up to 6th day. Seemed to be doing well until 7th day. Sclerae icteric	Blood cultures: negative 4th day, positive 6th day. Autopsy — gray consolidation of all lobes except part of apices. Dry pleural cavity.

TABLE VI—CONT'D

20	♂ 70	?	None								Day of death Index: 11.5 Direct reaction: positive Bilirubin: 1.1	Died 5th day. Entire right lung consolidated. Temp. 101. Pulse 100-120. Resp. 40.	Postmortem taps — no fluid in either pleural cavity W.B.C. 15,500; polys 85%
21	♂ 32	III	5th to 9th day	3	10 c.c. Amber Turbid	12.5	Neg.	0.8	N. G.		From 3d day Index: 15, 10, 13.6, 6, 6, 6.6, 3.8, 4 Direct reac.: pos. to 9th day, neg. Bilirubin: 1.25, 0.62, 0.52, 0.46, 0.33, 0.44, 0.31, 0.23	Acutely ill until 6th day. Delirrium tremens. Left lower lobe. Sclerae icteric	Blood cultures negative 4th to 7th days.
				8	8 c.c. Amber Turbid	12.5	Neg.	0.6	N. G.				
22	♂ 50	IX	8th to 12th day	10	10 c.c. Yellow Turbid	5	Del. 1	0.35	Type IX		From 5th day Index: 6.3, 5, 11.6, 10, 5, 3.3, 4.3 Direct reac.: del. 3, del. 4, pos. to 9th day, del. ½, neg. Bilirubin: 0.5, 0.41, 0.61, 0.83, 0.22, 0.25, 0.22	Lysis terminated on 10th day	Blood cultures negative 5th to 8th days W.B.C. 22,600 8th day W.B.C. 15,000 15th day Kahn and Wassermann positive
				12	Dry								
23	♂ 49	III	5th to 15th day	4	5 c.c. Wash-out of cavity all polys						From 4th day Index: 12.5, 15, 10, 7.5, 10, 6, 7.5 Direct reac.: pos. to 8th day, del. 8, neg. Bilirubin: 1.1, 0.93, 0.6, 0.45, 0.4, 0.4, 0.5	3d attack. Treated with antimeningococcic serum 6th day. Lysis terminated 7th day. Resp. 30 to 13th day. R. middle lobe. Fluid developed after tap (x-ray). Tap for fluid anlage	Blood cultures negative 4th, 5th days. W.B.C. 9,250; polys and metamyelocytes 92% on 4th day. 6,200 on 9th day
24	♂ 50	I	3d to 20th day	6	Air Left base						From 2d day Index: 12.5, 13.6, 8.3, 7.5, 10, 10, 8.3, 8.8, 6, 7.5, 4, 4, 5, 6 Direct reac.: pos. to 13th day, del. 3, neg. Bilirubin: 1.0, 0.7, 0.7, 0.7, 0.77, 0.86, 0.56, 0.6, 0.42, 0.4, 0.26, ∠.2, ∠.2, ∠.2	Specific antibody therapy 2d and 3d days fluid found by x-ray at R. base on 3d day, at left base 14th day. Fluoroscopy showed calcified interlobar pleura left. T.P.R. septic to 18th day.	Blood cultures negative 2d to 6th days. W.B.C. 35,700; polys 92% 2d day
				16	Air Left base								
25	♂ 56	III	8th to 14th day	8	1 c.c. Yellow Turbid						From 7th day Index: 6.5, 10, 10.8, 8.3, 7.5, 5, 4.3, 3.8, 3, 3, 3, 3.8, –, 3 Direct reaction: pos. to 18th day, –, neg. Bilirubin: 0.6, 0.6, 0.9, 1.2, 0.71, 0.6, 0.3, 0.21, 0.22, 0.23, 0.32, 0.3, (.2), /.2	Septic lysis to 24th day. Irrational to 14th day. Fluid obtained on 17th day by entering 9th space on right posterior below lower lobe, guiding needle point by contact with diaphragm to spot on dome.	Lung puncture 14th day no growth. Blood culture negative 11th day. W.B.C. 11,950; polys 84% 7th day. W.B.C. 15,250; polys 90% 9th day. W.B.C. 13,700; polys 88% 18th day
				14	Dry								
				17	2 c.c. Yellow Clear								

TABLE VI—CONT'D

SERIES NO.	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGNS OF FLUID	CHEST TAPS					KINETICS OF SERUM ICTERUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA
				DAY OF TAP	DESCRIPTION OF FLUID	ICTERUS IN FLUID: ICTERUS INDEX	ICTERUS IN FLUID: DIRECT REACTION	ICTERUS IN FLUID: BILIRUBIN	FLUID CULTURE			
26	♂ 42	I	10th to 18th day	10	Dry					From 8th day Index: 5, 10.8, 10, 12.5, 6.8, 6.8, 7.5, 6, 6 Direct reac: positive to 14th day, neg., neg. Bilirubin: 0.32, 0.9, 0.77, 1.0, 0.52, 0.5, 0.4, 0.25, ∠.2	Treated with specific antibodies on 10th and 11th days. Septic lysis terminated 20th day. Sclerae icteric Left lower lobe.	Blood cultures positive 8th to 10th days: negative 7th and after 10th days. W.B.C. 6,800—14,000 8th-9th days. Kahn and Wassermann positive
				12	25 c.c. Amber Turbid	12.5	Pos.	0.7	N. G.			
				15	50 c.c. Amber Clear				N. G.			
27	♂ 47	III	None	7	Dry					From 7th day Index: 8.3, 12.5, 11.8, 15, 16.6 Direct reaction: positive throughout Bilirubin: 0.5, 1.0, 0.9, 1.0, 1.05	Died 11th day. Right lower lobe. Temp. 98.6 at death following dose of types I and II antibodies. Sclerae icteric	Blood cultures positive daily—1,500 col. per c.c. on 10th day. Type III from postmortem lung puncture. W.B.C. 8,800 to 9,400; polys 92%
				10	Dry							
28	♂ 47	II	7th to 10th day	7	2 c.c. Yellow Turbid				N. G.	6th day 10th day Index: 10.7 16.6 Direct reac.: neg. pos. Bilirubin: 0.5 1.54	Died 10th day. Temp. 100.5 at death entire right lung consolidated. Sclerae icteric.	Blood culture positive 7th day. W.B.C. 14,500; polys 88% 7th day. Autopsy—gray consolidation of all right lung and left upper lobe; left lower red. R. pleural cavity 250 c.c. fluid; left 150 c.c.
29	♂ 45	I	None	8	Dry					From 5th day Index. 15, 30, 30, 16.6, 8.3, 8.3, –, 6.2, 6 Direct reac.: positive to 12th day, neg. Bilirubin: 0.57, 1.84, 1.6, 0.84, 0.25, 0.4, –, 0.23, 0.25	Right middle lobe only (x-ray). No signs in chest after 8th day. Rusty sputum to 6th day. Temp. fell to normal 7th day. Sclerae icteric	W.B.C. 10,000 5th day. Spectroscopic examination of serum on 6th-7th days showed only obliteration of the blue-violet and bands of oxyhemoglobin
30	♂ 38	I and VIII	6th to 8th day	7	2 c.c. Amber Turbid				Type I	From 6th day Index: 7.5, 6, 6.8, ----, 21.4, 30 12.5, 10.8, 11.5, 8.3, –, 6, 6, –, 5 Direct reac.: del. 4, del. 6, del. 3, ----, positive to 20th day, del. 2, –, neg. Bilirubin: 0.58, 0.25, ∠.2, ----, 0.83, 1.0, 1.0, 0.6, 0.5, 0.4, –, 0.3, ∠.2 – /.2	Nonspec. protein therapy 6th and 7th days. During recovery from type I infection became irrational and toxic on 12th day with type 8. R. lower lobe. True crisis on 14th day. Sclerae icteric	Blood cultures negative 6th, 7th, and 13th days. W.B.C. 11,000 on 7th and 23d days
				9	Dry							

TABLE VI—CONT'D

31	♂ 24	II	5th to 9th day	5	5 c.c. Amber Turbid	10	Pos.	0.65	No organisms	From 4th day Index: 18.7, 10.8, 15, 13.6, 7.5, 6, 6, – – –, 6, – –, 6 Direct reac.: positive (weak on 17th day) Bilirubin: 1.3, 0.6, 0.55, 0.35, 0.33, 0.33, 0.3, – – – ∠.2 – – ∠.2	2d attack. Specific antibody therapy 3d, 4th, 5th days. Lysis terminated 10th day. Right middle lobe. Fluid at base. Three week attack of "catarrhal jaundice" terminated month before. Skin jaundice	Blood cultures positive 3d day, negative thereafter
32	♂ 25	II	From 7th day	8	15 c.c. Yellow Turbid	7.5	Pos.	0.75	N. G.	From 3d day Index: 16.6, 21.4, 15, 10, 9.3, 10, – – 5, – 5, 19th-20th days 8.3, 5. 28th day 7.5. 38th day 7.5. 42d day 5. Direct reaction: positive to 38th day. Negative 42d day Bilirubin: 1.7, 1.8, 1.7, 0.66, 0.3, 0.33 – – 0.43 – 0.33. 19th-20th days 0.35, 0.25. 28th day 0.25. 38th day 0.2. 42d day ∠.2	Skin jaundice. Specific antibody therapy on 3d to 5th days. Lysis terminated 8th day. Otitis media (R) on 13th day—culture of hem. streptococcus from ear on 16th day. Sharp rise of temp. to 105 on 18th day—septic lysis following 8 wks. Erysipelatoid erythema spread over back from site of 3d chest tap, and "butterfly" developed over nose and face. Rib-resection 27th day. Transfusion on 28th day. Left chest. Another empyema developed on 30th day near hilum on right side, which drained by coughing through eroded bronchus.	Blood cultures positive for Type II on 2d day only, and for hemolytic streptococcus on 18th day only. W.B.C. 18,000; polys 85%; Hgb. 80% 3d day. W.B.C. 11,600 17th day. W.B.C. 14,000 22d day (polys 70%). W.B.C. 12,000 23d day. R.B.C. 1,700,000 Hgb. 60% 26th day
				13	30 c.c. Amber Turbid	6	Pos.	0.6	N. G.			
				22	130 c.c. Yellow Turbid	7.5	Neg.	0.45	Hem. Strep.			
				24	600 c.c. Yellow Turbid 1.2 c.c. Sediment in 10 c.c.	10	Neg.	0.4	Hem. Strep.			
				26	550 c.c. Flocculent 4 c.c. Sediment in 10 c.c.	6	Neg.	0.31	Hem. Strep.			
33	♂ 58	?	None							From 6th day Index: 33.3, 43 Direct reaction: positive throughout Bilirubin: 2.7, 2.6	Died 7th day. Intense skin icterus. Temp. 103-101. Pulse 110-130. Resp. 28-35. Enlarged liver.	W.B.C. 6,800. Autopsy—gray consolidation right upper lobe. Fibrinous exudate. Dry pleural cavity. Luetic liver and alcoholic cirrhosis
34	♂ 50	VII	None							From 5th day Index: 25, 30, 50, 75 (100 in p.m.), 100, 50, 37.5, 30, 15, 12.5, 10; 12.5 20th day Direct reaction: positive throughout Bilirubin: 2.0, 2.8, 3.7, 5.0 (7.0 in p.m.) 7.5, 3.3, 1.8, 1.0, 1.25, 0.75, 0.6; 0.56 on 20th day	2d attack. True crisis on 9th day. Sputum dark olive green, obviously bile laden on 8th and 9th days. X-ray showed consolidation of right middle lobe on 7th day. On 11th day x-ray completely negative—no physical signs persisted. Grandfather was negro. Patient a Sicilian.	Blood cultures negative 8th and 9th days. W.B.C. 34,000; polys 93% 6th day. W.B.C. 22,850; 11th day

Spontaneous sudden termination of the fever, followed by uneventful recovery, was not uncommonly observed in the first few days of illness without specific treatment when fluid was present. (8) All but type I or II patients treated early with Felton's concentrated antibodies recovered with pleural fluid. In the fatal case fluid was also present.

GENERAL CHARACTERISTICS OF THE ICTERUS OF LOBAR PNEUMONIA

The data given in the clinical protocol of the Boston City Hospital series shows the fluctuations of the pneumonic icterus in specific cases. This icterus is not to be regarded as specific for pneumococcus pneumonias, for it has also been observed in three cases due to Friedlander's bacillus, one dying at the index 25, another at 60, another with effusion. Since Friedlander's bacillus is not known to be bile-soluble, it is obvious that the icterus cannot be interpreted in the same way as for the pneumococcus. Since the hemolytic streptococcus and the staphylococcus do not, per se, produce pathologically the picture of lobar pneumonia, although they may produce clinical signs simulating lobar pneumonia, and they also are not bile-soluble, icterus again cannot be regarded in the same light as when the pneumococcus is the invader.

The zoning of the pneumonic icterus on the basis of the icterus index rather than on the quantitative bilirubin, alone makes possible a seemingly consistent classification. The van den Bergh reaction usually becomes direct positive at some time during the acute course of any lobar pneumonia, but does not afford a method of classification. In all cases terminating fatally, with the exception of two dying during the first four days of illness, the direct positive reaction has been present on the day of death. Following recovery without fluid (true crisis) it has persisted direct positive for several days. Accompanying recovery with fluid it gradually weakens, then frequently suddenly disappears completely, becoming negative in ten minutes without going through the development of and progressive lengthening of the delay period of the delayed reaction. Delayed resolution with fluid appears to be measured by the duration of this direct positive reaction, even though the icterus index and quantitative bilirubin have already dropped to normal.

The quantitative bilirubin, per se, cannot be used as a basis for classification, but exhibits certain striking characteristics in its relation to the icterus index in the white race, particularly when the reaction curves are associated with the true crisis phenomenon (recovery without fluid). This mechanism accompanying true crisis has proved difficult to isolate for satisfactory study because it seems quite rare and the changes may take place so rapidly that they may elude the twenty-four hour determinations. However, in some slower cases it has been evident, and it corresponds closely to a similar reaction occurring during the ascent of icterus often encountered in familial jaundice, pernicious anemia, the newborn, and occasionally following a traumatic interstitial blood extravasation.

The characteristics of this as yet rather vaguely understood phenomenon (the riddle of the icterus index 16.6) are as follows: (1) As the icterus index ascends in the absence of a direct positive van den Bergh reaction, it is halted

in its rise at 16.6, while at this point the quantitative bilirubin increases sharply to disproportionately high levels. (2) As the icterus index rises from 16.6 to 30, a definite downward loop in the quantitative bilirubin takes place, which may continue downward with the rising index, or may recover from its fall to regain at 30 approximately the value it had attained at 16.6. (3) In familial jaundice, pernicious anemia, and in the newborn the rise of the icterus index above 16.6 is usually accompanied by the development of an anomalous type of direct positive van den Bergh reaction, an immediate golden accentuation (instead of the typical reddish or amber color). In lobar pneumonia, however, should this golden reaction actually be present, it is completely masked by the invariable presence of the reddish direct positive reaction. (4) The negro race has not as yet been found to exhibit this phenomenon. Thus far it appears to be associated with icterus only in the white race. (5) Icterus ascending with a direct positive van den Bergh reaction does not exhibit the 16.6 phenomenon in its complete form, for either the halt at 16.6 is prolonged (as in many fatal cases of lobar pneumonia in the white race), or else as 16.6 is passed, no subsequent downward loop in serum bilirubin content is manifest (except in lobar pneumonia in the white race). The icterus of lobar pneumonia in the white race, then, exhibits the same characteristics in its changes as are exhibited in the type of icterus known as "hemolytic" except for the fact that the van den Bergh reaction is direct positive.

The phenomenon just described is undoubtedly the crucial factor in the consideration of, and approach to, the problem of bilirubin therapy in lobar pneumonia. It is also quite obvious that should intravenous injections of bilirubin (in 4 per cent sodium hydroxide) effect a change in the icterus index zones, and thereby possibly affect the outcome, unless it be used with all the data at hand, it might as readily result in a fatality in a case which would otherwise have recovered spontaneously, as it might induce recovery in an otherwise fatal case, since, depending on the efficiency of the fluid mechanism, it is essential to *pass* the icterus indices 10 or 16.6, and in case 30 should be exceeded, to reach 100. Exactly how much pigment is necessary to force these icterus index zones remains to be determined. The explanation of why the zones should signify what they seem to is by no means clear, but it is probable that at the icterus indices 10, 16.6, and 100 some change takes place in the physical state of bilirubin as it concentrates in a serum medium, manifested by marked disturbances in the relation of the quantitative bilirubin to the icterus index at these points, and due to changes in the size of suspended aggregates of free nascent colloidal bilirubin, which does not give any reaction in the aqueous (direct) van den Bergh test rather than to the bilirubinate which gives rise to the direct positive reaction, and it is probable that these two forms are present together. Although pure bilirubin is now being used routinely by several individuals intravenously as a liver function test only up to 1 mg. per kilo of body weight, it is indicated by Emerson[10] that it could be used in greater amounts without any harmful effects.

Barjot,[2] Castellanos y Gonzales,[6] and Ziegler[34] have used bile salts intravenously in spite of their known toxicity with apparently very favorable results

in pneumococcus infections, and it may well be considered whether or not the bile salts play any rôle in the icterus index zones. This conjecture, however, cannot be confirmed or refuted until a suitable simple method is devised for the quantitative determination of the bile salt content of whole blood,[25, 27, 29] for, unlike bilirubin, they are found in normal red cells[27] as well as in the plasma.

RATIONALE

I. *The Icterus Mechanism.*—Analysis of the cases observed since the beginning of this study indicates that approximately 30 per cent of hospitalized patients having lobar pneumonia do not form any fluid in their pleural cavities, and face a mortality of from 75 per cent to 80 per cent. Recovery in such cases appears to depend exclusively upon the movements of their serum icterus. Obviously bile-solubility should be analyzed as fully as possible. Atkin[1] found that if pneumococci were cultured so that they contained no autolysin, they were not bile-soluble, and concluded that bile-solubility was but an acceleration of the normal autolytic process of these organisms, analogous to the action of bile on any autolytic process. In the consolidation of lobar pneumonia, which is the most striking feature of localization in the human organism in response to pneumococcus invasion, the outstanding biochemical phenomenon is likewise autolysis. Cleavage products are much in evidence, arising from this consolidated area, leucocytic enzymes playing a prominent rôle in their production,[33] although they are inhibited in this rôle by blood serum,[16] which also inhibits autolysis.[16] Pneumonic sputum contains peptones, proteoses, and amino acids, and acquires a marked peptolytic activity after "crisis"[16]; albumoses appear in more than 60 per cent of the cases in blood or urine[16]; during and after the fastigium, the urine contains an excess of organic acids[14]; there is an increase in noncoagulable nitrogenous substances in the blood,[33] and after crisis, the serum contains an enzyme acting specifically on pneumococcus protein, accompanied by an increase in serum ereptase.[33] Weiss[32] found by lung punctures that there was insufficient undigested pneumococcus protein in consolidated areas to produce any anaphylactic phenomena in sensitized animals. Lung punctures have also shown that in cases evolving favorably no living pneumococci could be obtained after the fifth day,[21] although clinical recovery did not take place until later. It appears, then, that the organisms in their pulmonary foci are undergoing autolysis, hence their two fractions, the haptene (specific soluble substance of Avery and Heidelberger) and the common pneumococcus protein are split from one another,[35] the former neutralizing artificially injected specific antibodies, and the latter stimulating the formation of natural antibodies specific only for pneumococcus protein or its cleavage products,[15] and type specific antibodies, of spontaneous origin, would hardly be expected to appear in any appreciable concentration.

It has been found that pneumococcus autolysis at first produces a highly toxic proteose, but that if this autolytic process is carried on beyond the proteose stage, all toxic properties disappear.[26] It is significant that Rosenow[26] treated 200 cases of lobar pneumonia by the intravenous injection of autolyzed

pneumococci (sufficiently autolyzed so that most of the toxic substance had disappeared), with a mortality of 3 per cent in 95 cases treated within forty-eight hours, and of 11 per cent in 105 cases treated on or after the third day.

The question now arises, "What particular constituent of bile accelerates autolytic processes?"; and specifically, "What constituent might accelerate the autolytic process of lobar pneumonia beyond the stage of toxic cleavage products?" Tatum[30] found that whole bile was more effective, although less penetrating, than the bile salts alone, in accelerating tissue autolysis, and also observed that bile with its bilirubin content removed was less effective than whole bile. It is generally assumed that the bile salts are the substance active in producing pneumococcolysis, but Kelly[18] and Sellards[28] have found that whole bile is more reliable, since different strains of pneumococci vary in their susceptibility to lysis more with the salts than they do with whole bile. Sellards could obtain no evidence that bilirubin, known to be cytolytic in the presence of light, was at all pneumococcolytic in a 1 per cent alkaline solution in the test tube.

Experiments in animals frequently give results different from those observed in the test tube. Koslowski[19] has pointed out there are present in bile numerous unconjugated higher soaps which are far more pneumococcidal in vitro than sodium taurocholate or sodium glycocholate. On the other hand, Lamar,[20] in showing that pneumococci could be destroyed by sodium oleate, observed that although sodium linoleate and sodium linolenate were more effective in this action in test tube experiments, the reverse held true in animals, for serum completely inhibited the action of the more unsaturated soaps, and the greater the degree of unsaturation, the less was the protection afforded the animal. The evidence that the pigment bilirubin is primarily involved in the relation of jaundice to the outcome in lobar pneumonia is derived entirely from the study of patients, and is sufficiently consistent to suggest, as a provisional hypothesis, that the autolytic process of lobar pneumonia is accelerated by a change in the physical state of bilirubin, associated chiefly with "the riddle of the icterus index 16.6" whereby a form of the pigment evolves which acts as a catalyst on this autolytic process, as well as acting destructively on all autolysin-containing pneumococci; that until and unless this change takes place, the pigment has no favorable effect upon the disease or the organisms, except in cases where it is acting in conjunction with the effusion mechanism. It is of interest that from many medical centers during the influenza epidemic of 1918 type-specific pneumococci were frequently reported to be bile-insoluble[7, 17, 24] and therefore not subject to autolysis.

In the negro race the findings indicate that at the icterus index 100 a phenomenon analogous to, but not identical with, that which takes place in the white race at 16.6, occurs. White patients who exceed the icterus index 30 develop curves which may be interpreted only as negroid reactions. From the phylogenetic point of view it is possible that these curves do not always indicate negro ancestry (although such ancestry was disclosed in the only case in which inquiries were made), but may often be due to other racial mixtures.

II. *The Fluid Mechanism.*—In approximately 70 per cent of the cases observed in this study there was evidence of the presence of a fluid pleural exudate, and in this group the mortality was only 15 per cent. Effusions were often demonstrable before the icterus index had dropped to normal, thus affording data as to the zone entered and forecasting the outcome. However, in those cases found presenting icterus indices below 10 when first seen, no data were available to show whether the 11-15 zone had been entered or not, and in such cases the outcome could not be forecasted with accuracy. In this latter group the van den Bergh reaction was closely followed, and danger of death remained as long as the direct positive reaction persisted.

A description of the pulmonary lymphatic system makes it possible to understand the mechanics of pleural fluid formation. Gage[11] states that the lymphatic drainage of each lobe is so constructed that the peripheral portion of the lung parenchyma is drained toward the pleural surface, whence collecting lymphatic channels run subpleurally to the hilum. It is probable that this system cares for the drainage of a large portion of the alveoli, for they are not reached by the deeper or peribronchial and perivascular system.[13] In the presence of an inflammatory process, shutting off the lymphatics of a lobe, the slightest change in the permeability of the walls of these lymphatic channels would result in a fluid pleural exudate. Furthermore, Gorecki[12] points out that carbon particles injected intratracheally in dogs later are found on the pleural surfaces, a finding again consistent with the operation of this peripheral pulmonary lymphatic system. Should consolidation take place rapidly, fluid mobility would be checked by the dehydration of the lobe involved, and no fluid could form in the pleural cavity.

This fluid mechanism seems analogous to drainage, coupled with the extraordinary properties of the constituents of pleural effusions contributing to the destruction of pneumococci. Often when such drainage occurs promptly all that can be found in the chest is a small effusion persisting for such a short time that it may easily be completely overlooked, while others, due to their long duration, finally compel the clinician to diagnose them, although without cell studies they may readily be confused with the effusions of tuberculosis. The successful operation of the effusion mechanism appears to depend largely on three factors: (1) unimpeded autolysis of pneumococci, (2) phagocytosis and the action of leucocytic enzymes, (3) the bilirubin content of the fluid (dependent on the serum level). The greater number of these fluids are sterile, even during the acute course of the disease, adequate evidence of their pneumococcostatic or pneumococcocidal power. Duyck,[8] working on pneumonia in the natives of the Belgian Congo, found that a few drops of such pleural fluid rendered a broth culture of virulent pneumococci innocuous to susceptible animals with many times the ordinary lethal dose.

The rôle of white cells (polynuclears, clasmatocytes, monocytes) in the successful operation of the effusion mechanism, correlated with the bilirubin content of the fluid appears of paramount importance, for favorable cell immigration and emigration was observed only in the fluids of higher bilirubin content. Since the fluids obtained in the Boston City Hospital series were

submitted to Drs. Scott and Forkner of the Thorndike Laboratory for cell studies and to Dr. Finland for serologic studies, the detailed description of this part of the work will be left to them. Cells were sufficiently numerous in fluids from recovery cases to cause them to appear turbid, grossly like empyemata, but microscopically showing active living cells in vital preparations. The various phases of cell activities observed in successive taps, and the changes associated with empyema will be discussed by Drs. Scott and Forkner. During the recession of the serum icterus the bilirubin content of the fluids remained high, and receded very slowly, indicating the slowness of reabsorption from the pleural cavity, thus affording a possible means of estimating the maximum intensity of the icterus previously attained.

In tapping for pneumonic effusions it was often noticed that a marked drop in the fever followed, more frequently than could be ascribed to coincidence. None of the cases tapped early continued prolonged febrile reactions during convalescence. On the other hand, in many cases, tapping seems not at all necessary.

The site for tap is obviously wherever the signs of fluid[5] are found, often the costophrenic angle in the posterior axillary line, and frequently more mesially posteriorly in the midscapular line or closer to the spine. Interlobar pockets are best diagnosed by x-ray. It should be borne in mind that the fluid is usually in a thin sheet between lung and chest wall,[22] and that a shallow tap is necessary. Tapping, if contemplated for diagnosis or for the study of fluids, should be done as early as possible, since many fluids do not persist longer than a few days. The duration of fluid exudates in the pleural cavity has been stated by Gorecki to be proportional to their colloid content, since the digestive action of enzymes is required before such substances can leave the pleural cavity. Withdrawal of fluid soon after its formation removes these colloids (proteins), thus facilitating reabsorption and reducing to a minimum residual adhesions from the organization of fibrin.

A dry tap, unless repeated at different sites, is not conclusive evidence against the existence of fluid, but the obtaining of it in any amount is positive evidence, for ordinarily the pleural cavity contains only enough moisture to lubricate the pleural surfaces, and not enough to be obtainable by tap.

A chest needle, fitted directly on a 50 to 100 c.c. syringe, has proved most satisfactory, since the resistance to the progress of the needle point may be palpated by the rigidity of the instrument, and it is quite easy to detect the entry into the pleural cavity, contact with the diaphragm, or the yielding touch of lung surface. Care should be taken to make all movements in line with the shaft of the needle, even when searching laterally in the space between lung and chest wall. With the proper use of novocain chest-tapping caused little or no pain, except in an occasional patient with a thick chest wall. The asepsis was essentially the same as that used in routine lumbar puncture.

Normal icterus indices, when found after the fourth or fifth day of illness, have in the adequately studied cases been significant of the existence of an effusion, but in such cases a prognosis cannot be made on the basis of the

normal index. Bernheim[4] found in a series of cases that those with normal icterus indices did not die, and although her findings are confirmed by a large number of noninfluenzal cases in this series, there have also been several discouraging exceptions. In the normal icterus index group the van den Bergh reaction has proved the test of value, for these patients are decidedly not out of danger until even the weakest possible direct positive reaction has disappeared. Since such cases are often not studied until late in the course of the illness, no data are available as to whether or not they had previously exceeded the icterus index 10 without examination of the pleural fluid.

SUMMARY

Serial studies in 224 cases of lobar pneumonia by the daily determination of the icterus index, van den Bergh aqueous (direct) reaction, and the quantitative serum bilirubin estimation have demonstrated that serum icterus manifested in one or all of the three tests, is pathognomonic of the disease. The changes in intensity and character of this icterus may be closely correlated with the events of the pneumonic fastigium, their interpretation depending on (1) the determination of the maximum icterus index attained, and (2) the determination of the presence or absence of a fluid pleural exudate arising from each lobe involved. In the absence of such fluid the icterus index tends to oscillate upward; in the presence of fluid it tends to return to normal. Fluid pleural exudates were found or indicated in 70 per cent of the cases studied, and in this group the mortality was 15 per cent. In the 30 per cent without fluid, however, the mortality was 75-80 per cent. The total series mortality was 37.2 per cent.

In the absence of fluid, in the white race, the mortality was 100 per cent with icterus indices up to and including 16.6. In cases entering and not exceeding the icterus index zone 18.7-30 there were no deaths, and the day of crisis corresponded with the attainment of the maximum icterus index. Following the drop of the index during crisis, it remained above normal for several days. A corollary study of icterus in other diseases and conditions implies that in ascending jaundice the icterus index 16.6 is a biologic constant in the white race, and that in the absence of a direct positive van den Bergh reaction (hemolytic jaundice) a change in the physical state of bilirubin occurs as it is passed, this change manifesting itself by a downward loop in serum bilirubin as the index rises in the zone 18.7-30. In lobar pneumonia, although the van den Bergh reaction is direct positive, this same phenomenon takes place, and appears to be associated with recovery.

In negroes, in the absence of fluid, the icterus index 16.6 is of no significance, and recoveries occurred only when the index 100 was exceeded. When, in the white race, the icterus index 30 was exceeded, recoveries then occurred (by true crisis) only if the index 100 was attained, thus manifesting a reaction essentially negroid in character.

In the presence of pleural fluid (70 per cent of cases), in the white race, no deaths occurred in the icterus index zone 11-15. In many cases, however, constantly exhibiting icterus indices at or below 10 during the period of

observation it could not be ascertained whether or not they had previously entered this zone, and the estimation of the outcome depended on the determination of the changes in the van den Bergh reaction, recovery being indicated by the confirmed disappearance of the direct positive reaction. In the negro race, insufficient cases have been observed to make definite conclusions when fluid is present, although no deaths have as yet been observed in the icterus index zone 11-30.

The prognostic value of the study of the icterus in lobar pneumonia should not, perhaps, be unduly stressed, since each case exhibits its own individual characteristics and often the trend of the icterus is not discoverable until the clinical indications of the outcome are clearly manifest. Frequently, however, such a study furnishes a means for the estimation of the outcome in doubtful cases.

The summation of the findings relating to the pneumonic icterus clearly indicates that a therapeutic trial of bilirubin is the logical conclusion of the investigation, particularly since the pigment is believed to be nontoxic when injected intravenously. Although the analysis of the icterus of lobar pneumonia may seem complex because of its relation to the presence or absence of fluid and because of the differences between the white and black races, the application of these findings to the therapeutic test is simple enough, and may be stated as follows:

1. In the white race inject a quantity of bilirubin sufficient to cause the icterus index to *pass* 16.6, but not to exceed 30, regardless of the presence or absence of fluid. (It is possible that to exceed 30 may not be detrimental in pure Caucasian stock.)

2. In negroes, and when negroid reactions occur in the white race, the icterus index 100 must be attained.

3. By preliminary studies correlating the serum icterus with the physical findings the addition of surplus bilirubin may be contraindicated in many cases.

4. Additional data on the bilirubin content of pleural fluid, obtained early in the course of the disease, in its relation to the outcome, should be secured.

The writer wishes to express sincere appreciation of the thorough and scientific manner in which much bacteriologic data were obtained by the members of the Pneumonia Service at the Boston City Hospital, and for their splendid cooperation in studies pertaining to the proper evaluation of some of the pathologic phenomena of lobar pneumonia. The results with specific serum therapy were favorable.

Studies on the physical chemistry of pure bilirubin, obtained from the Chemisch-Pharmazeutische A. G., Bad Homburg, Werk Frankfurt, a. M., Germany, in so far as they have as yet been carried out, are confirmatory of the significance of the icterus index constants encountered in lobar pneumonia from the point of view of colloid chemistry.

REFERENCES

1. Atkin, E. E.: Rationale of Bile-Solubility of the Pneumococcus, Brit. J. Exper. Path. 7: 167, 1926.
2. Barjot: Sur un traitement des septicemies pneumococciques par l'injection intraveineuse de sels biliaires, Bull. Acad. de med., Paris 100: 898, 1928.
3. van den Bergh, A. A. Hijmans: Der Gallenfarbstoff im Blute, 2d Ed., S. C. van Doesburgh, Leiden, and Johann Ambrosius Barth, Leipzig, 1928.

4. Bernheim, Alice R.: The Icterus Index: A Quantitative Estimation of Bilirubinemia, J. A. M. A. **82**: 291, 1924. Bilirubinemia: Significance of Variations, Arch. Path. & Lab. Med. **1**: 747, 1926.
5. Cabot, Richard C.: Physical Diagnosis, p. 330, ed. 9, Wm. Wood & Co., 1928.
6. Castellanos y Gonzáles, A.: Prioridad de Cuba en el tratamiento quimioterápico-biológico de las pneumococcias por las sales biliares, Rev. de med. y cir. de la Habana **34**: 133, 1929.
7. Dunn, A. D.: Observations on an Epidemic of Bronchopneumonia in Omaha, J. A. M. A. **71**: 2128, 1918.
8. Duyck: Formes cliniques des pneumococcies tropicales au Congo Belge: leur traitement par les ferments leucocytaires des épanchements pleuraux, Rev. gen. de clin. et de therap. **42**: 276, 1928.
9. Elton, N. W.: Icterus Index Studies in Lobar Pneumonia, J. Mich. State Med. Soc. **27**: 818, 1928. Latent Jaundice of Lobar Pneumonia, J. Mich. State Med. Soc. **28**: 451, 1929. Icterus Index Studies in Lobar Pneumonia II: Relationship of the Icterus Index Curves to Crisis and Fluid Pleural Exudate, New England J. Med. **201**: 611, 1929. Serum Pigmentation: Kinetics of the Latent Jaundice of Lobar Pneumonia, J. Detroit Coll. Med. & Surg. **1**: 34, 1930.
10. Emerson, W. C.: The Toxic Constituent of the Bile, J. LAB. & CLIN. MED. **14**: 635, 1929.
11. Gage, Simon H.: Lymphatics of the Lung, Reference Handbook of the Medical Sciences **8**: 405, Wm. Wood & Co., 1889.
12. Gorecki, Z.: Quelques remarques sur le méchanisme de la formation des épanchements pleuraux, Rev. de med., Paris **45**: 361, 1928.
13. Gray, Henry: Anatomy of the Human Body, ed. 20, Lea and Febiger, 1918.
14. Holten, C.: Formation of Organic Acids and Retention of Chloride in Lobar Pneumonia, Arch. Int. Med. **38**: 489, 1926.
15. Jobling, J. W., Strouse, S.: Immunization With Proteolytic Cleavage Products of Pneumococci, J. Exper. Med. **16**: 860, 1912.
16. Jordan and Falk: The Newer Knowledge of Bacteriology and Immunology, Univ. of Chicago Press, 1929.
17. Keegan: The Prevailing Pandemic of Influenza, J. A. M. A. **71**: 1051, 1918.
18. Kelly, F. B.: Solution of Pneumococci by Bile, Am. J. Pub. Health **10**: 708, 1920.
19. Kozlowski, A.: Comparative Studies of the Action on Pneumococcus of Bile Acids and Unsaturated Fatty Acids found in Bile in the Form of Soaps, J. Exper. Med. **42**: 453, 1925.
20. Lamar, R. V.: Action on Pneumococcus and Its Experimental Infections of Combined Sodium Oleate and Anti-serum, J. Exper. Med. **13**: 1, 1911. Further Observations upon the Action of Certain Soaps on the Pneumococcus and Its Experimental Infections, J. Exper. Med. **14**: 256, 1911.
21. Mair, W., and McLeod, J. W.: A System of Bacteriology **2**: p. 164. Medical Research Council, His Majesty's Stationery Office, London, 1929.
22. McCrae, Thomas: Early Diagnosis of Empyema in Lobar Pneumonia, Clinic of Thomas McCrae, Jefferson Hospital, Med. Clin. N. A. **12**: 883, 1927.
23. Neufeld: Ztschr. f. Hyg. u. Infektionskrankh. **34**: 454, 1900.
24. Nuzum, J. W.: Pandemic Influenza in a Large City Hospital, J. A. M. A. **71**: 1562, 1918.
25. Perlzweig, W. A., and Barron, E. G.: Bile Acids in Blood: Quantitative Colorimetry, Proc. Soc. Exper. Biol. of Med. **24**: 233, 1926.
26. Rosenow, E. C.: Partially Autolyzed Pneumococci in the Treatment of 200 Cases of Lobar Pneumonia, J. A. M. A. **70**: 759, 1918.
27. Rosenthal, F.: Wislicki, L.: Biliary Acids in Jaundice, Klin. Wchnschr. **6**: 781, 1927.
28. Sellards, A. W.: Selective Action of Dilute NaOH on Certain Races of Pneumococcus, J. A. M. A. **71**: 1301, 1918.
29. Szilard, P.: Colorimetric Method for Quantitative Determination of Bile Salts in Blood, Biochem. Ztschr. **173**: 440, 1926.
30. Tatum, A. L.: Influence of Bile on Autolysis, J. Biol. Chem. **27**: 243, 1916.
31. Thannhauser, J. S., Andersen, E.: Bilirubin in Blood Serum, Deutsch. Arch. f. klin. med. **137**: 179, 1921.
32. Weiss, C.: Biochemical Studies of Pneumonic Exudates, Arch. Int. Med. **23**: 395, 1919.
33. Wells: Chemical Pathology (Autolysis in Pneumonia), ed. 5, pp. 80-82, W. B. Saunders, 1925.
34. Ziegler, E. E.: The Specific Effect of Bile Salts on Pneumococci and on Pneumococcus Pneumonia, Arch. Int. Med. **46**: 644, 1930.
35. Zinnser, H.: Immunologic Considerations of Pneumonia and Discussion of Rational Basis for Vaccine Therapy, New England J. M. **200**: 853, 1929.

ADDITIONAL REFERENCES

von Bergmann, G.: Functional Pathology of the Liver, Klin. Wchnschr. **6**: 776, 1927.

Elton, N. W.: Physiology, Correlations and Technic of the Van den Bergh Reaction, Icterus Index, and Quantitative Serum Bilirubin, J. LAB. & CLIN. MED. **17**: 1, 1931.

Friedman, J. C., and Straus, D. C.: Bilirubin Determinations in Cholecystitis Without Jaundice (Observations in Lobar Pneumonia), J. A. M. A. **82**: 1248, 1924.

Fukuda, J.: Sterilizing Function of the Liver Against Pneumococci, J. Orient. Med. **8**: 21, 1928.

Harris, B. R.: Alterations in Liver Function as Index of Toxemia in Pneumococcus Lobar Pneumonia, J. Clin. Investigation **4**: 211, 1927.

Harrop, G. A., Jr., and Barron, E. S. G.: Excretion of Intravenously Injected Bilirubin as a Test of Liver Function, J. Clin. Investigation **9**: 577, 1931.

Jankelson, I. R., and Gargill, S. L.: Bilirubin Liver Function Test. I. Modification of the Method, New England J. M. **204**: 547, 1931.

Ravdin, E. G.: Estimation of the Clinical Value of the van den Bergh Test (Observations in Lobar Pneumonia), Am. J. Med. Sc. **169**: 850, 1925.

THE EFFECT OF ULTRAVIOLET IRRADIATION ON GLUCOSE SOLUTION*†

By L. M. Dillman, B.S., Chicago, Ill.

THE effects of ultraviolet irradiation on glucose solutions as referred to in an earlier[8] paper prompted a further investigation as a preliminary to the study of the effects of the procedure on carbohydrate metabolism.

Berthelot and Gaudechon[2] found that 10 per cent glucose was quantitatively decomposed to carbon monoxide, methane and hydrogen after ten hours' exposure to a quartz mercury vapor lamp. Neuberg[6] on the other hand found no change in rotation after exposing 10 per cent glucose solutions to sunlight over a period of two months or longer but the solution became less dextrorotatory after three days if ferrous sulphate was added. Euler and Lindberg[3] irradiated a 10 per cent glucose solution with a quartz mercury vapor lamp at 75° and found that gases were evolved after two hours; after twelve hours the gases were identified as methane, hydrogen, carbon monoxide and carbon dioxide. Lowry and Courtman[5] found that irradiation for thirty minutes with a quartz mercury vapor lamp did not accelerate isomeric changes.

A possible explanation of these conflicting reports is the lack of standardized conditions of time of exposure, source of radiation and voltage, the distance of the solution from the source of energy, the temperature of substance as well as its P_H, and the presence of salts or catalysts, every one of which is variable and yet determines to some extent the course of the reaction.

The object of the present investigation was to determine the nature of the change in glucose solutions after irradiation, since most reports indicate some changes. It was, at first, intended to use some one of the uric acid methods for analysis, since in this way a constant standard could be employed, i.e., the amount of uric acid reduced. Due to the variability of the reduction of phosphotungstic acid by dilute glucose solutions the standard methods were out of the question (Table IV).[8] These methods were modified by using different concentrations of Na_2CO_3, NaOH, and other alkalies but the results were unsatisfactory. Micromethods for blood sugar were not considered because these entailed determining the percentage of change in reducing power and not a constant standard.

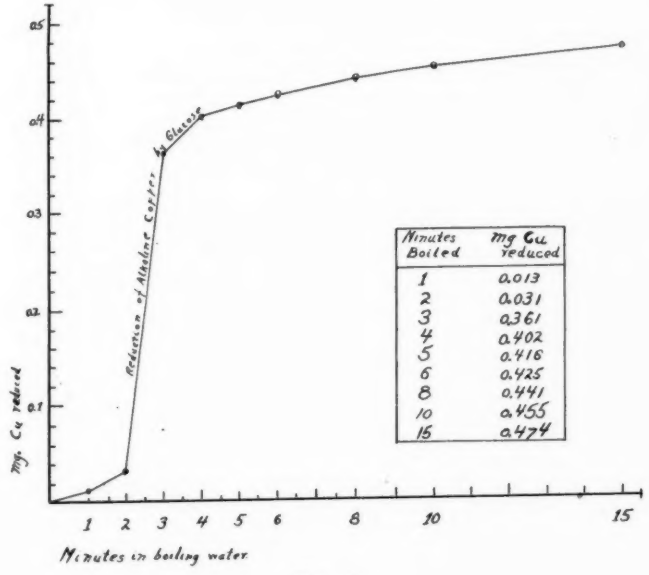
In a few preliminary experiments it was noted that the total reducing power of the glucose solution did not change, whether it was irradiated or not, according to the Folin-Wu[4] micromethod, Benedict[7] micromethod, or Shaffer and Hartmann[7] method. Since the mechanism of the oxidation of glucose by alkaline copper is not perfectly understood, it is logical to suppose that the

*From the Department of Physiology, University of Illinois, College of Medicine.
Received for publication, July 2, 1931.

†This investigation was financed in part by a grant from the Graduate School; in part by a grant from the Committee on Scientific Investigation of the American Medical Association; in part by a grant from the Phi Rho Sigma Medical Fraternity.

glucose might be so rearranged and slightly decomposed that the mixture produces the same total reduction as the freshly prepared glucose solution. If, now, we determine the reduction of the mixture (glucose-alkaline copper) at intervals of one minute, and plot a curve of this reaction, a mixture of decomposition products giving the same total reduction would, perhaps, show a different curve of reduction.

The curve of the reduction of alkaline copper by glucose was worked out as follows: 5 c.c. of the Shaffer-Hartmann citrate reagent*[7] and 5 c.c. of glucose solution (Merck's c. p., dried 24 hours at 85°), containing not more than 2.0 mg. of glucose were pipetted into a large test tube and placed in a boiling water-bath for a certain time (indicated in Table I), cooled, acidified with 5 c.c. of H_2SO_4 and titrated with 0.005 $Na_2S_2O_3$, starch used as indicator.



Minutes Boiled	mg Cu reduced
1	0.013
2	0.031
3	0.361
4	0.402
5	0.416
6	0.425
8	0.441
10	0.455
15	0.474

Fig. 1.

Results of the averages of at least four determinations are shown in Fig. 1. Titration agreed surprisingly well considering that we stop a reaction while proceeding at a fair rate of speed. After three minutes' boiling, the titrations checked within 0.1 c.c. Reduction occurs as soon as the tube and contents have reached the temperature of the water-bath. The first part of this reaction, the first three minutes, is the stage in which we would expect to find a deviation for a decomposition mixture. This was accomplished by reducing the temperature.

The experiment was repeated, except that the temperature was maintained at 70° to 75°. This time in the water-bath as indicated in Fig. 2. There is a "latent" period before any noticeable reduction occurs.

*$CuSo_4$—5 gm. per liter; Na citrate—16 gm. per liter; KI—10 gm. per liter; KIO_3—0.7 gm. per liter; $K_2C_2O_4$—18 gm. per liter. The copper is reduced by the glucose and on acidifying, iodine is liberated which oxidizes the cuprous oxide, according to the equation $2Cu^{++} + 2I = 2\ Cu^{+} + I_2^{-}$. The excess iodine is titrated with thiosulphate.

Aqueous solutions of Merck's c. p. Glucose dried 24 hours at 85° (solution prepared the same day as used) were irradiated in a fused quartz flask (5 mm. in thickness) at a distance of one inch (at this distance there was uniform diffusion throughout the flask) with a water cooled Kromayer quartz mercury arc lamp at room temperature. (21°-23°.)

TABLE I

DEGREES DEXTROROTATION

PER CENT GLUCOSE		TIME IRRADIATED				
0		15 min.	30 min.	60 min.	90 min.	5 hr.
0.1	0.103		0.103	0.107	0.103	
0.5	0.52		0.49	0.49	0.49	
1.0	1.07		1.14	1.07	1.11	
5.0	5.23	5.2	5.26			5.26
10.0	10.71	10.74	10.74			10.76
15.0	16.16					16.08
20.0	21.41					21.48
50.0	55.09					55.29
50.0	55.36					55.23

Solutions 0.1 per cent, 0.5 per cent, 1.0 per cent were made up fresh and three drops of 0.2 per cent NH_3 added before measured.

Solutions 5.0 per cent to 50 per cent were made up and set in refrigerator for two weeks or longer.

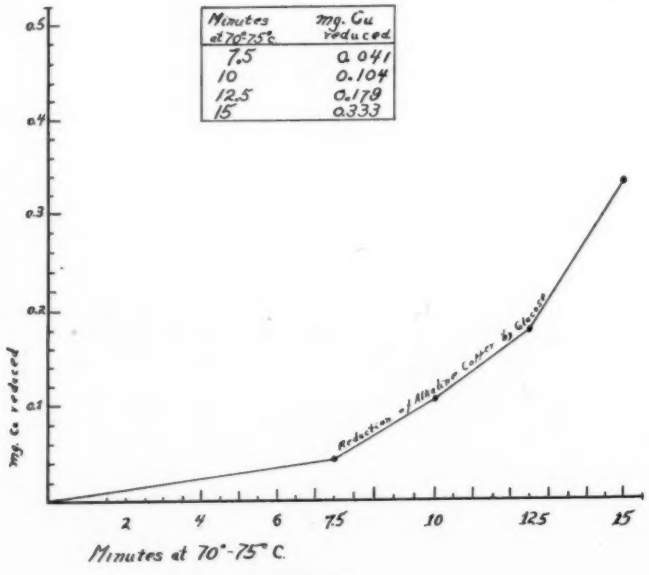


Fig. 2.

The reduction curve, or parts of the curve, was determined for the following:

1. 0.1 per cent irradiated 30 minutes
2. 0.1 per cent irradiated 60 minutes
3. 0.1 per cent irradiated 90 minutes
4. 1.0 per cent irradiated 30 minutes
5. 1.0 per cent irradiated 60 minutes
6. 1.0 per cent irradiated 90 minutes
7. 1.0 per cent irradiated 3½ hours

Complete curves were worked out in Cases 1, 2, and 3 but are not reproduced because they agree in every particular with the curves in Figs. 1 and 2.

In Cases 4, 5, 6, 7, only the most prominent points of the curves were plotted, namely, boiling water-bath for four and fifteen minutes and at 70° to 75° for three, six, and nine minutes. In none of these cases was there any divergences from curves shown.

It was thought that perhaps the reagent was too strongly alkaline to detect any slight change that might be present, so the experiment was repeated (the most prominent points of the curve being plotted), using the original citro-carbonate reagent to which had been added 0.1 M. boric acid. This solution slowed the reaction, but the curves of the normal and of the irradiated solutions did not differ. Two-tenths M. boric acid was now added to the original citro-carbonate reagent which further slowed the reaction but did not produce any change in the form of the curves.

By the method employed we can determine quite accurately any change in reducing power that may be produced in a given solution, but in view of the negative results there was a question whether any change had been produced in the glucose solutions, so polariscopic measurements were made to determine the rearrangement.

Fresh solutions of glucose in distilled water were prepared as described above, irradiated for thirty, sixty, and ninety minute intervals and the rotation determined, after addition of 3 drops of 0.2 per cent NH_4OH. Results are shown in Table I. It is obvious that there is no rearrangement in any of the solutions, all readings being within the limit of error. No change in optical rotation is observed in 20 per cent glucose after five hours' irradiation.

Two samples of 50 per cent glucose solution showed no change in rotation after five hours' irradiation, although in one sample, gas bubbles were produced after four hours.

CONCLUSIONS

By the methods employed, reduction of alkaline copper solution and polariscopic measurements, it was impossible to determine any change in glucose solutions when irradiated with quartz mercury vapor lamp.

Thanks are due Dean A. H. Daniels of the Graduate School, to the committee on Scientific Investigation of the American Medical Association, and to the Council of Phi Rho Sigma Medical Fraternity for financial assistance.

REFERENCES

1. Benedict, S. R.: The Estimation of Sugar in Blood and Normal Urine, J. Biol. Chem. **68**: 739, 1926.
2. Berthelot, D., and Gaudechon, H.: Sur le Mécanisme des Réactions Photochimiques et le Formation des Principes Végétaux; Decomposition des Solutions Sucrées, Compt. rend. Acad. d. Sc. **151**: 395, 1910.
3. Euler, H., and Lindberg, E.: Über Biochemische Reaktionen in Licht, Biochem. Ztschr. **39**: 410, 1912.
4. Folin, O., and Wu, H.: A Simplified and Improved Method for Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.
5. Lowry, T. M., and Courtman, H. R.: Dynamic Isomerism XV Influence of Light on Isomeric Change, J. Chem. Soc. **103**: 1214, 1913.
6. Neuberg, C.: Chemische Unwandlungen Durch Strahlemarten, Biochem. Ztschr. **39**: 158, 1912.
7. Shaffer, P. A., and Hartmann, A. F.: The Idometric Determination of Copper and Its Uses in Sugar Analysis, J. Biol. Chem. **45**: 365, 1921.
8. Dillman, L. M.: The Effects of Ultra-Violet Irradiation on the Reducing Power of Blood, J. Lab. & Clin. Med. **17**: 45, 1931.

STUDIES IN THE ALIMENTARY CANAL OF MAN*

IX. The Calculation of Gastric Volume†

By W. A. Sommerfield, M.D., Cleveland, Ohio

INTRODUCTION

IN THEIR studies upon gastric reaction patterns Miss Kuenzel and Doctor Todd, in a series of papers, have demonstrated the effect of different stimuli upon the behavior of the stomach. They have shown, for instance, that, following buttermilk, the roentgenographic shadow is longer, broader, and of larger area than after a milk meal of the same bulk and under the same conditions. They have shown that warming the abdominal wall or administration of a hot milk meal is followed by a shadow of less dimensions and smaller area than that resulting from a milk meal of the same bulk at 70° F. administered without application of heat.

Continuing this important line of investigation the authors mentioned have shown that shadow area is modified in the same subject following the administration, in equal bulk, of water or lactic acid, and after application of cold. Further they have been able to demonstrate that the characteristic shadow area is exaggerated by previous administration of a preliminary meal, a curious phenomenon which has been termed facilitation.

Comparison of linear dimensions of gastric shadow has its defects for the method of measurement must necessarily utilize, as measuring points, standardized arbitrarily selected sites upon the shadow contour. A standardization of this type can take no account of the variations in disposition of the stomach which the presence of the other abdominal viscera induce by altering its tilt or position from time to time and even during a serial roentgenographic study. It is also difficult to discount the fluctuations in shadow pattern necessarily accompanying peristalsis and purely local changes in the Magenblase.

As a check upon the interpretation of linear dimensions Miss Kuenzel used the planimeter record of shadow area but apart from the error of 6 per cent which she claims is inevitable in these records[8] the shadow area, though permissible as an index of volume, gives no real assurance of the volume which, after all, is what we really seek to obtain.

Linear dimensions and area of roentgenographic shadow are of value, utilized in the manner and for the purpose described by these authors, in whose hands the records so obtained have permitted a quantitative interpretation of roentgenoscopic observations on gastric activity. But these methods have been

*From the Anatomical Laboratory, Western Reserve University.
Received for publication, June 9, 1931.
†The Steuer Prize dissertation in Anatomy, Western Reserve University 1930.

employed temporarily pending a time when more reliable or appropriate technical devices should be made available. It is my purpose therefore to describe an alternative, and possibly a more satisfying, method of analysis of the roengenographic record. I shall discuss the technique, apply it to the elucidation of gastric problems and seek to give the method its validation in examining critically the results obtained in the light of information secured along other lines.

CURRENT ESTIMATES OF STOMACH CAPACITY

Observations on adult stomach volume have been made by Luschka, Ewald, Ost and Kelling.

Luschka[5] used dead stomachs, blew them up moderately so as not to stretch their walls, drained them of contents and then filled them with water. In seeking to determine gastric form by filling the organ in situ with plaster Luschka recognized that he had also an alternative method of obtaining volume.

Ewald[2] used Luschka's method but attained no more satisfactory results because he merely forced water under pressure into the dead stomach.

Ost[6] modified this technique to some extent in permitting water to flow by its own weight alone into the stomach instead of by pressure from behind. But Ost also investigated the living organ, filling it in the same way by allowing water to flow in until the patient complained of pain and then abstracting the water by suction, or forcing in air under pressure with the same end in view.

Kelling[4] forced air into the living stomach to a prearranged pressure and then, detaching the stomach tube from the pump, collected the air regurgitated.

All these methods are so obviously crude and inaccurate that it is hardly necessary to dwell on them. In summary they are indirect measurements made during life and direct determinations after death. Both depend essentially upon the introduction of fluid under artificial and uncontrollable conditions. Other authors have pointed out that the amount of gas or liquid escaping through the pylorus or lodging in the esophagus is not ascertained. There are objections even more significant than these in the living. We have as yet no precise information concerning the reflex effect of introducing a rubber tube into the stomach. Motility, secretion and size, we find, may be affected by direct reflex or cerebral stimulation. There is probably a marked complication from the secretion of gastric juice and our roentgenoscopic observations warn us that this secretion may be both considerable and rapid.

Measurement of volume in the dead stomach has still more obvious defects, for the organ is atonic or, if the cadaver be embalmed, may present bizarre forms in shape or volume as was demonstrated long ago by Cunningham.[1] As a matter of fact we may eliminate at once the estimates based upon measurement of autopsy specimens or those older records drawn from the determination of volume upon dead organs embalmed by arsenic, alcohol, or other reagents before the introduction of formalin.

Nevertheless gastric volume is currently conceived to lie somewhere between 1500 and 3000 c.c. Vierordt[9] has gathered together the various estimates which are summarized in Table I.

Taking a series of 10 stomachs at random from adult male bodies embalmed by a mixture of alcohol, carbolic acid, and glycerin with about 5.45

TABLE I

ESTIMATES OF STOMACH CAPACITY IN C.C. OBTAINED BY INTRODUCTION OF FLUIDS

	DEAD		LIVING	
	AIR	WATER	AIR	WATER
Luschka		Male 2500 to 2600 Female 1750		
Ewald	250-1600		1600-1700	
Ost			1830-2700	2267-2533
Rosenheim			1700	
Kelling			587-1300	1300-1700

per cent of formalin I have obtained the results presented in Table II. The organs after excision were ligated on the duodenal side of the pylorus and a pursestring suture was tied around the esophageal orifice into which a nozzle was inserted. The stomach was carefully filled with water which was then emptied into a graduated cylinder. The average volume of stomachs 2 to 10 inclusive all showing formalin contraction was 563.5 c.c. So small is the figure in comparison with that currently quoted that one might reasonably doubt the validity of the method which is undoubtedly complicated by the formalin injection. A single specimen of flaccid stomach gave a capacity of 1263 c.c. but even this figure is smaller than most accepted averages.

TABLE II

CAPACITY OF ADULT MALE STOMACHS IN C.C. REMOVED FROM FORMALIN-EMBALMED BODY AND WATER FILLED

NO.		NO.	
1	1263	6	405.5
2	684.5	7	405.5
3	474	8	629
4	541	9	609
5	473	10	850
	Average 633.45 c.c.		

THE MATHEMATICAL DETERMINATION OF STOMACH CAPACITY

Having failed to obtain anything like the usual figure by filling the dead stomach with water and suspecting current estimates of exaggerating the actual capacity we decided to seek a method of determining volume from the roentgenographic picture of the living stomach. For this purpose the ordinary anteroposterior roentgenogram is not sufficient: we must also observe the gastric shadow in the lateral roentgenogram. Fortunately a number of such records had been made by Doctor Todd and Miss Kuenzel. From these, one notes that the stomach after a small meal is a cylindrical tube slightly flattened from before backwards. The flattening varies somewhat even in the same individual from time to time but for practical purposes, within the lim-

its of determination possible, this variation may be ignored. We then measured the shadow of a barium-filled stomach projected upon the roentgenoscopic screen, and having equalized, as well as possible, the distance between stomach and screen in both lateral and anteroposterior postures, found the gastric tube shadow just below the Magenblase 85 mm. broad in both anteroposterior and lateral roentgenograms. Though this line of investigation must be methodologically unsatisfying it taught us, at least, that the stomach after a small meal may, for practical purposes, be considered a cylindrical tube and thus our expectation that the roentgenographic shadow could be used as a basis of estimating stomach volume received encouragement.

It has already been stated that hitherto, in this laboratory, the area of gastric shadow has been measured by the planimeter. But it might have been done more accurately, though with greater expenditure of time, by the application of Simpson's rule. I do not purpose to trace the derivation of this rule from the analytic expression of a parabolic curve. For that the reader should refer to any favorite exposition of the calculus. There is a very clear account in Gibson's little volume.[3] Simpson's rule is the following: if an area under a curve of unknown analytic expression is to be determined, divide this area into an equal number of strips by equidistant ordinates. Then find the sum of the extreme ordinates, plus twice the sum of the odd ordinates, plus four times the sum of the even ordinates. Multiply this quantity by the fraction obtained by taking one third of the distance between ordinates.

Clearly a calculation so involved as that just enunciated is not worth while in practice unless one can be fairly sure of its approximation to a true valuation of volume, especially as the planimeter reading can be made in a fraction of the time. In our preliminary survey of shadow areas made upon several hundred roentgenograms, when we could hope only for rough approximation, the planimeter served admirably. By its help Miss Kuenzel and Doctor Todd have differentiated size of shadow area under different conditions as I have already stated. These investigators have therefore been able to forecast more or less roughly the probable differences to be found in stomach volume under different experimental conditions. The application of Simpson's rule becomes imperative to complete our study, now that it is possible to make the necessary corrections in dimensions of shadow area.

AN ILLUSTRATIVE EXAMPLE

With the human stomach as our example of a tube of irregular contour, and a tracing of the projection of its outline on a roentgenogram as our basis for calculation, assuming the tube to be circular in cross-section, diameters at equidistant intervals are measured off and the areas at these levels calculated from the formula $\frac{1}{4}\pi D^2$.

When the distance between ends of summit of Magenblase and lowest point of greater curvature, measured parallel with the vertebral column, is divided into ten equal intervals and the capacity calculated by Simpson's rule, we find that the result differs by only 4 c.c. from the capacity computed when twenty such intervals are used. Hence we have restricted our calculations to the ten-interval basis.

To obtain ten equal intervals, eleven ordinates are drawn at right angles to the parallel just defined. The first and eleventh of these, being tangents to the gastric outline, give areas of zero value, hence there are nine areas to compute. The stomach volume is then found by adding twice the sum of the areas derived from the odd ordinates to four times the sum of the areas derived from the even ordinates and dividing the total by one third of the distance between two successive ordinates.

THE ARTIFICIAL TEST STOMACH

In the course of the work a suggestion was given that perhaps more accurate results might be obtained if one drew the ordinates perpendicular to the axis of the stomach. In order to compare the probable reliability of the

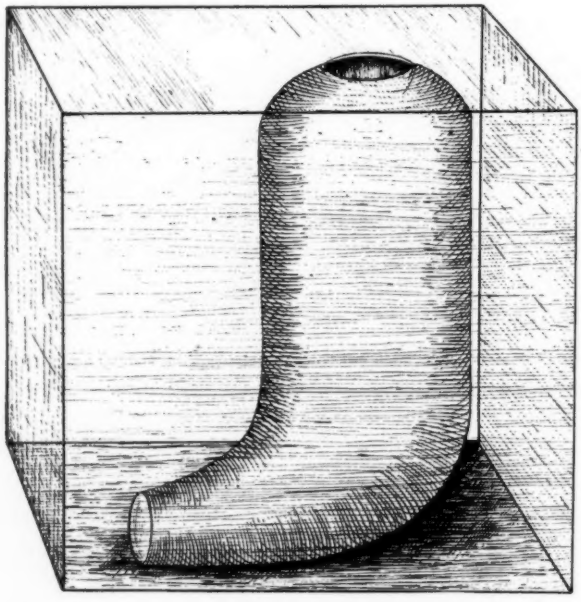


Fig. 1.—Plaster mould manufactured from model of artificial stomach. See text.

suggested technique with the method already described an artificial stomach was cast in plaster. This rough model of an average stomach was scraped down to even dimensions (see Fig. 1) and its volume measured. From it, a plaster mould was constructed and the two halves fitted exactly together leaving a small opening above to permit the mould to be filled with water. Before fitting the halves together the interior was thoroughly coated with shellac so that it would be water-tight.

From the cast model a set of calculations were made by each method. Finely adjusted calipers graduated in half-millimeters were used to determine the diameters at the different levels. All measurements were checked twice. The first method detailed above gave a volume of 580.56 c.c. and the suggested alternative method gave one of 493.81. Then the mould was filled with water and emptied into a 1000 c.c. graduate cylinder. The actual vol-

ume was found to be 580 c.c. Not only does this prove that the first method is correct but that it is accurate within experimental and calculable limits.

The source of error in the suggested alternative method lies in the fact that measurements based on a curved axis do not apply to a formula calculated on Cartesian coordinates. In order to develop a formula for the curved axis of the stomach from Magenblase to pylorus one would have to use polar coordinates and conical sections. This would be far too cumbersome, and there is no expectation that it would prove to be more accurate than the simple application of Simpson's rule for plane surfaces to the calculation of volumes.

THE CORRECTION FOR OBLIQUITY

The technique just described leaves out of account the distortion of shadow outline produced by obliquity of the stomach. Hence a correction must be made in the ordinates actually measured on the tracing of the roentgenogram. Inasmuch as the upper part of the stomach lies further from the photographic film than the lower part its shadow area is correspondingly greater. Hence we must know the approximate forward inclination of the organ upon what is called the stomach bed. For this the lateral roentgenogram is advisable though not absolutely necessary since the slope of stomach may be sufficiently accurately calculated by considering the stomach itself as the hypotenuse of a right-angled triangle of which the side is the vertical height of stomach shadow measured on the tracing parallel with the vertebral column and the base is one half the anteroposterior diameter of the patient at the level of the Magenblase. If this diameter has not been actually taken, it may be assumed to be 35 mm. for a slender male subject, and 50 mm. for one of stout (pycnic type) build.

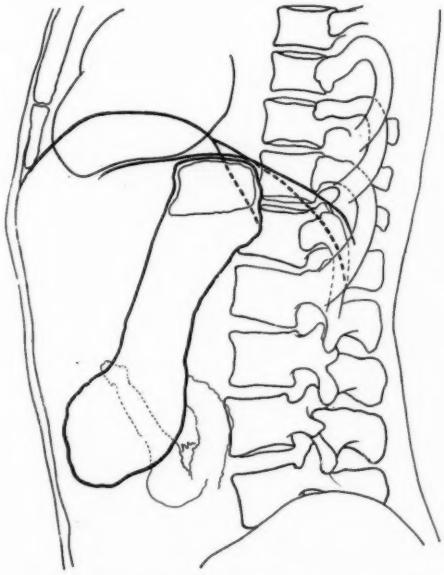


Fig. 2.—Lateral roentgenogram showing oblique relation of stomach to anterior abdominal wall.

Since the rays spread from the anode in the form of a long cone it follows that the breadth of shadow at any given level will bear the same relation to the actual stomach diameter as the distance of photographic film from the target bears to the distance of that gastric plane from the target. Having obtained and plotted the stomach obliquity towards anterior abdominal wall it is easy to compute the distance of any particular stomach level (or ordinate) from the anode because the distance between target and photographic film is known. The necessary correction of the ordinate measurement on the tracing is then made by simple proportion. When all the corrected ordinate values have been computed one may proceed to the application of Simpson's rule. In order to put this method to a practical examination the following technique was devised. The obliquity of a test stomach was first computed from a lateral roentgenogram (Fig. 2). A piece of card cut at this angle is

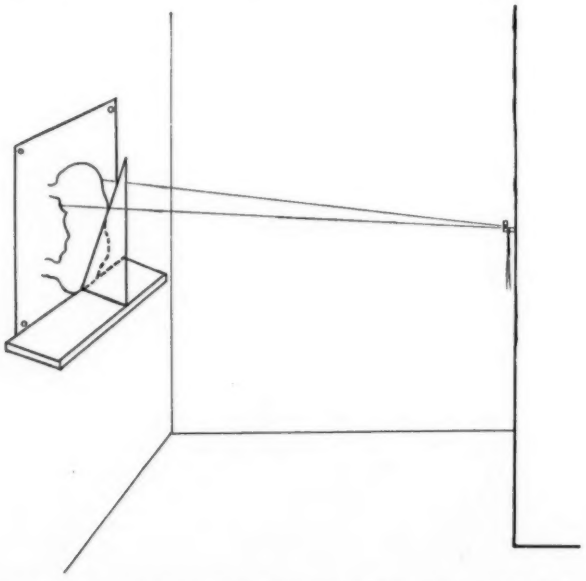


Fig. 3.—Apparatus for checking computed corrections of shadow diameters (ordinates). Target placed approximately at level of disc between first and second lumbar vertebrae. Strings with needles attached to stomach outline at extremities of an ordinate. Cardboard marker reproducing obliquity of stomach. By a steel rule measurement is made of the distance between the threads at the card hypotenuse.

mounted at right-angles to a stomach tracing, its lower angle just touching the lower margin of greater curvature on the tracing. Two threads representing divergent rays, each having a small needle at its free end, are attached to a support representing the target and placed at the target distance from the photographic film. The needles attached to the threads are then inserted into the traced stomach outline at the extremities of successive ordinates (see Fig. 3). The distance between the threads is measured by a steel rule placed against the sloping edge of the card and the observed distance checked against the computed distance.

As an illustration of the use of this method I have measured a series of forty roentgenograms of a single trained and stabilized stomach taken at intervals of twenty seconds after the swallowing of a meal composed of four

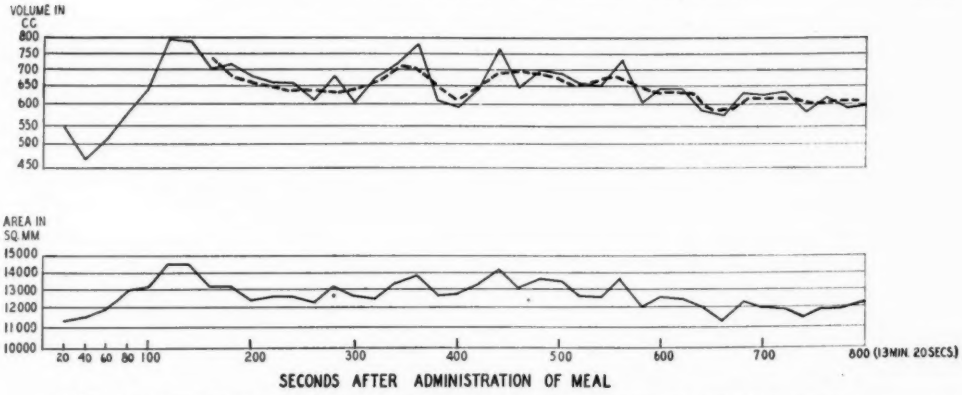


Fig. 4.—Comparison of stomach volume and shadow area on arithlog paper.

Diminution of volume and its rhythmic fluctuations are better seen in the graph of volume than in that of shadow area. Smoothing is obtained by averaging the determinations at successive levels. The first five roentgenograms are of little value for it is only in the sixth that the barium fully canalizes the stomach.

TABLE III

ROENTGENOGRAPHIC STUDY OF STOMACH AFTER 5-OZ. BUTTERMILK MEAL

SERIAL NO. IN STUDY (W.A.S.)	VOLUME C.C.	AREA SQ. MM.
1	541.37	11440
2	467.85	11560
3	507.40	11960
4	566.10	13060
5	636.34	13160
6	791.08	14580
7	784.15	14500
8	695.60	13140
9	710.10	13220
10	673.32	12460
11	658.97	12600
12	653.08	12640
13	610.83	12360
14	676.88	13280
15	603.78	12760
16	675.88	12530
17	715.58	13460
18	772.21	13870
19	607.05	12760
20	593.23	12820
21	639.18	13340
22	761.12	14280
23	645.34	13220
24	699.30	13640
25	693.07	13530
26	657.61	12720
27	653.36	12650
28	724.50	13550
29	604.44	11990
30	642.03	12580
31	643.15	12420
32	584.14	12100
33	572.88	11350
34	628.64	12280
35	627.43	11970
36	632.72	11970
37	578.14	11510
38	616.00	11890
39	596.40	11990
40	599.90	12100
Average	643.5 ± 7.26	Average 12506 ± 85.91
Standard deviation	68.06	Standard deviation 805

ounces of buttermilk with 33 grams of barium sulphate. In Table III are reproduced the successive determinations of capacity and the planimeter records of area. Fig. 4 gives the same information in graphic form.

THE REPRESENTATION OF GASTRIC BEHAVIOR

The plotting of calculated volume and measured area of shadow upon arithlog paper permits direct comparison of the two resulting curves. That for volume is given both in original computations and in smoothed form. It is apparent that both curves tell the same story. The volume curve has however more marked fluctuations than the curve of area. Naturally the fluctuations of both curves correspond accurately with each other in time relationship. The gastric volume falls for forty seconds after swallowing the meal. This is probably due to the loss of fluid through a patent pylorus. Then with closure of the pylorus the volume rises owing to outpouring of gastric juice. At the end of two minutes an adjustment occurs between flow of juice and rhythmic opening and closing of pylorus so that the volume falls rapidly at first and then progressively more slowly until, toward the end of the experiment, the reduction in volume becomes very gradual. These interpretations are based upon other experiments made by Doctor Todd, Miss Kuenzel and others of our staff upon gastric behavior. For further elucidation and explanation the reader must refer to other studies from this laboratory. It is apparent that the computation of volume by Simpson's rule gives a more vivid representation than simple shadow area of the details of gastric behavior. It does not actually bring any new inferences into evidence.

The first striking result of the experiment is the singular similarity in average volume of the stomach under investigation to the average volume of our dead stomachs 2-10 inclusive. This is of course not the average capacity after a hearty meal. What volume the stomach attains after a large meal is a problem for later investigation.

Another result probably of considerable significance is the rhythmic fluctuation in capacity as the stomach empties. This is very clearly shown on the smoothed graph.

The third point to which attention should be drawn is the comparative inadequacy of the first five roentgenograms since barium does not fully canalize the stomach until the sixth, namely two and one-third minutes after swallowing the meal.

It is of course obvious that this method of computation is open to objection. The stomach will not be a perfect tube. The peristaltic waves, in their passage detract somewhat from the reliability of the roentgenographic record. The exact distance of stomach from target is not known, nor is the obliquity of the stomach accurately registered. Valid as all these objections are, their total effect is but a small fraction of the disturbance in gastric behavior caused by the introduction of tube and of air or water.

One must make the reservation that the method here described will give much less reliable results in hypotonic stomachs which hang down in the abdominal cavity as flattened bags.

The probable usefulness of the mathematical method is evident in our routine laboratory studies and may be illustrated in a few sentences. Determination of area, as apparent from the graph, is subject to modification by irregularities of outline which have less effect upon the record of volume. The latter therefore shows more clearly a rhythmicity of pattern which appears to be characteristic of all our studies. Secondly, by the courtesy of my colleagues I am able to give in Table IV a brief record of other parallel studies made by the application of Simpson's rule in the manner which I have described in this article and illustrated in Table III. Studies in our laboratory have very consistently pointed to a slightly smaller gastric volume after five ounces of water than after five ounces of milk and to a considerably larger gastric volume than that characteristic of milk when the same quantity of buttermilk is administered. Lactic acid results have proved somewhat perplexing and are still being investigated. Very many serial studies indicate that the size and rhythmicity of gastric volume are produced by the interplay of gastric secretion and rhythm of pyloric opening and closing. What apparently erratic influence is at work in our lactic acid studies has not yet been determined.

TABLE IV

COMPARISON OF AVERAGE GASTRIC VOLUME (C.C.) IN EIGHT SERIAL ROENTGENOGRAPHIC STUDIES SHOWING INFLUENCE OF DIFFERENT STIMULI

	MILK	BUTTERMILK	WATER	LACTIC ACID
C.O.V. '32	286		254	
J.M.N. '30	318	400		
W.E.M. '31			476	418*
W.A.S. '31		643		810*

*The lactic acid results are so conflicting that some interfering cause is indicated. This is now being investigated.

In conclusion I desire to state that this work was undertaken during the tenure of a Crile Scholarship in the Anatomical Laboratory and I acknowledge my indebtedness to Doctor Wingate Todd for his help in preparing this manuscript for publication.

SUMMARY

1. Current estimates of gastric volume suffer from defective technique inasmuch as gastric behavior is influenced by the introduction of unusual foreign substances into the stomach and conditions into the experiment.

2. To obviate these difficulties of technique a method is here presented which requires simply an anteroposterior roentgenogram and a determination both of the obliquity of the stomach and the distance of the Magenblase from the anterior abdominal wall.

3. Simpson's rule, as applied to the volume of a tube of unknown curvature, provides a fairly reliable method of estimating stomach capacity under the particular conditions of our small meal experiments. It also provides a ready measure of the changing volume as contents become transferred to the small intestine.

4. The diminishing gastric volume shows rhythmic fluctuations which find their explanation in other studies.

5. Estimated by this method an illustrative five-ounce (150 c.c.) buttermilk meal in a healthy young adult stomach bespoke an average volume of only 643 c.c. when measured over the first thirteen minutes after swallowing. Since this is greater than the amount swallowed, the secretion of gastric juice must account for the difference.

REFERENCES

1. Cunningham, D. J.: Varying Form of the Stomach in Man and the Anthropoid Apes, Trans. Roy. Soc. Ed. 45: 1-47 (in reprint form), 1906.
2. Ewald, C. A.: Cited in Vierordt, p. 114.
3. Gibson, G. A.: Introduction to the Calculus, London, 1921.
4. Kelling: Physikal untersuchung über die Druckverhältnisse in der Bauchhöhle, Ztschr. f. Biol. 44: 219, 1903.
5. Luschka, H.: Cited in Ost.
6. Ost, A.: Beitrage zur bestimmung der capacität des Magens, Diss. Dorpat., 1891.
7. Rosenheim, T.: Cited in Vierordt, p. 114.
8. Todd, T. W., and Kuenzel, W.: The Attainment of Reliability in Gastric Responses, J. Lab. & Clin. Med. 14: 1010, 1929.
9. Vierordt, H.: Daten und Tabellen für Medicíner. 3 Auflage, 1906, Jena.

LABORATORY METHODS

MECHANICAL AIDS IN LABORATORY PROCEDURES*

By W. L. Robinson, Toronto, Canada

THE modern laboratory is becoming increasingly dependent upon its mechanical and technical equipment. Technicians, assistants, and professional staff, like the artisan, to do justice to themselves must be provided with proper tools. Time and thought spent on this phase of the work brings its reward in an efficient and smooth running routine.

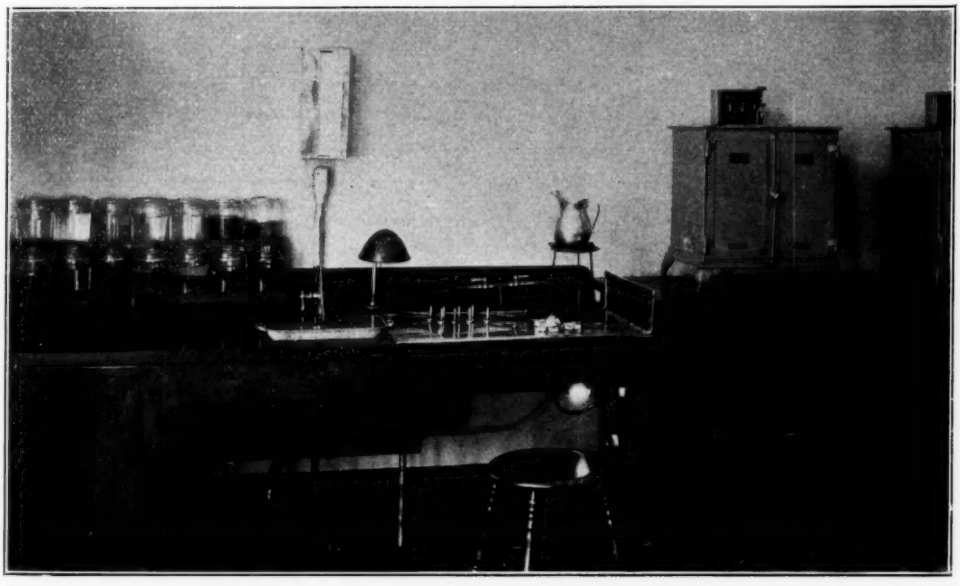


Fig. 1.—Shows complete installation of the hot paraffin plate. On the left are the jars of the deparaffining fluids and also the staining and dehydrating fluids. On the bench, in front of the jars, is our special slide rack used for carrying the sections through the various alcohols and dyes.

WARM PLATE FOR PARAFFIN EMBEDDING

Within recent years we have evolved and introduced into our laboratories pieces of apparatus which have proved to be very helpful in the carrying on of the day's work. The first, a warm plate for paraffin embedding has been found to be very useful and to facilitate to a great degree the paraffin embedding of tissue blocks. By our technic the blocks of tissue with respective labels are strung on a fine thread. These are carried through the alcohols into paraffin by the Autotechnicon machine. The string is then placed on

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Received for publication, June 26, 1931.

the warm plate, the blocks cut free with a pair of scissors and embedded in paper boats. This latter procedure is carried out on the warm plate kept at a constant temperature of 70° C. Its construction is simple, consisting as it does of a sheet of copper 18 × 36 inches with the edges rolled and corners soldered, except at one end where the sheet dips over into the sink. To the bottom of the sheet and 6 inches from the sink end there is soldered a copper box 4 inches square by 3 inches deep. A hole is cut through the copper sheet and a spout inserted to correspond to one corner of the box beneath. This is used to fill it with water. A heating element consisting of an ordinary plug-in electric heater used for hot water boilers is inserted in one side. A screw nut is sweated into the side of the box and the heater screwed in. In a similar

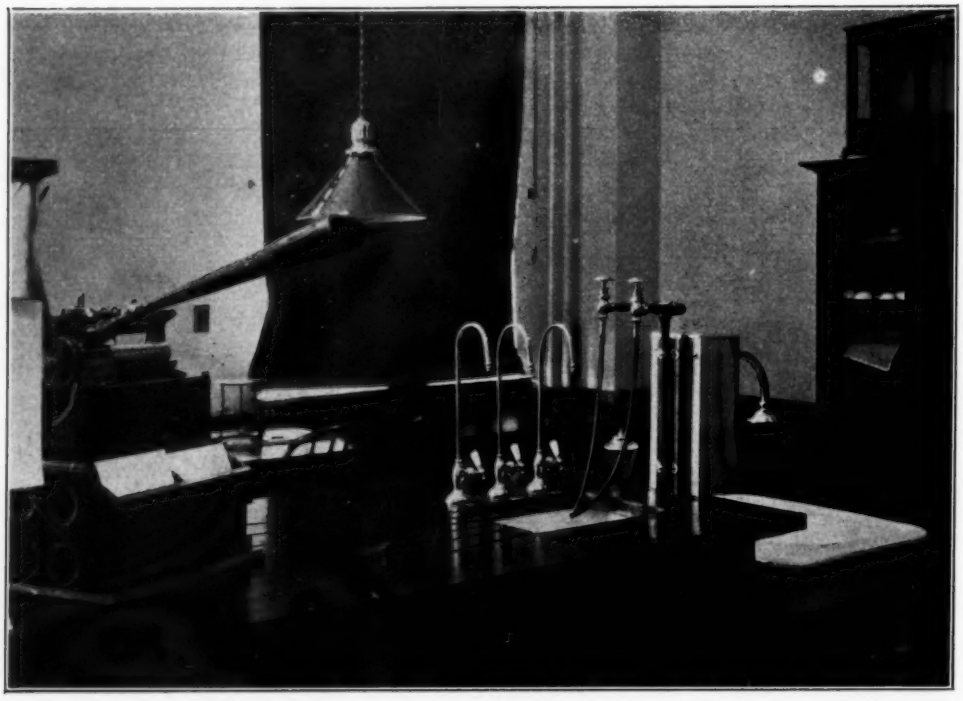

Fig. 2.—Completely equipped workbench for surgical gross material including dictaphone, sink, hot and cold water, special taps for formalin, alcohol, and distilled water, and at the end of the bench a surgeon's washbasin with knee controlled water supply.

manner an electric thermostat is inserted. These are connected to a floor plug and a switch with pilot light wired in the line. The complete installation, including the paraffin oven, sink, and staining jars, is shown in Fig. 1.

A PRESSURE SYSTEM FOR FORMALIN, ALCOHOL, AND DISTILLED WATER

Instead of the gravity system with its unsightly row of large bottles overhead we have installed a pressure system with ordinary water taps for distributing the formalin, alcohol, and distilled water as required. The large winchesters containing these fluids are placed in the locker space beneath the workbench. Air pressure is piped to the bench and a pressure reducing valve inserted in the line. The winchesters are closed with rubber corks with two glass

tubes running into each. One goes to the bottom, the other just through the cork. The latter is hooked up to the air pressure line, and the former by rubber tubing is connected to a goose-neck tap which drains into the sink as shown in the illustration (Fig. 2). The taps are of the ordinary quick action lever type. These are stock equipment which may be purchased from any plumber. We have found that an air pressure of three pounds is the most satisfactory, producing a good flow of fluid without much danger of blowing the corks out of the bottles.

MICROTOME KNIFE SHARPENER

The need for such a machine is evidenced by the many new devices reported for this purpose in the literature. The principle involved is not new. Its application for this purpose has worked out very satisfactorily. It consists essentially of two steel rollers 14 inches in length by 3½ inches in diameter with a shaft running through the center. Two six-inch wide bands

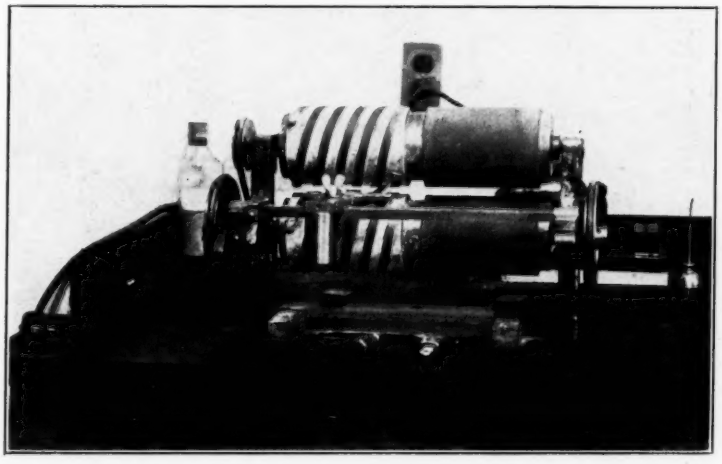

Fig. 3.—Front view of microtome knife sharpener showing the smooth leather rollers on the right for grinding and the spiral leather strap on the left for finishing. The large pulley at the left end actuates the cam shaft.

of heavy leather belting are cemented on to each roller. The band on the right end of the roller is built up until a thickness of about ½ inch is attained. The roller is then put in a lathe and the leather very carefully turned down until a thickness of about ⅜ inch is left. It is smoothed down with emery paper. The leather band on the left end of the roller is cemented on in the same manner until a thickness of about ¼ inch is attained. This is turned down in the lathe to ⅛ inch in thickness. A ¼ inch thickness of sponge rubber is then fitted over this and cemented to the underlying leather. Where the edges meet, a row of fine tacks are put in to prevent the rubber from curling. Over this are wound three carefully selected pieces of leather strapping ½ inch in width and $\frac{3}{32}$ inch in thickness. These are fastened at each end by drilling a hole into the steel roller, tapping it, and inserting a screw. The leather straps are wound around the roller in a spiral fashion. The other roller is prepared in exactly the same manner. The rollers are mounted in brass bearings and supported on a cast iron frame (Fig. 3).

A holder for the knife is shown in position attached to the rocker bar by a thumbscrew in Fig. 4. It is constructed of a metal bar $4\frac{3}{4} \times 1\frac{1}{2} \times \frac{1}{2}$ inches with a slit in the center for the screw-bolt to hold it to the rocker bar. On one end is fastened a counter-balance weight and on the other a metal jaw to hold the knife. This jaw fits into the first bar by a pin and socket joint so that it can rotate sideways and allow for an even pressure on the rollers.

The rocker bar is supported at each end in brass bearings and actuated by a cam located underneath at the left end (Fig. 3). The cam is rotated at a speed much less than that of the rollers by reduction gears. By this bar with its cam the knife is pressed first against one roller then the other. One turn is required to throw the knife from the one roller to the other. It is then held against the roller for three turns. It is our intention however to put in more gears to reduce the speed of the rocker arm shift so that the knife will be held in contact with the roller for some 12 to 14 turns each.

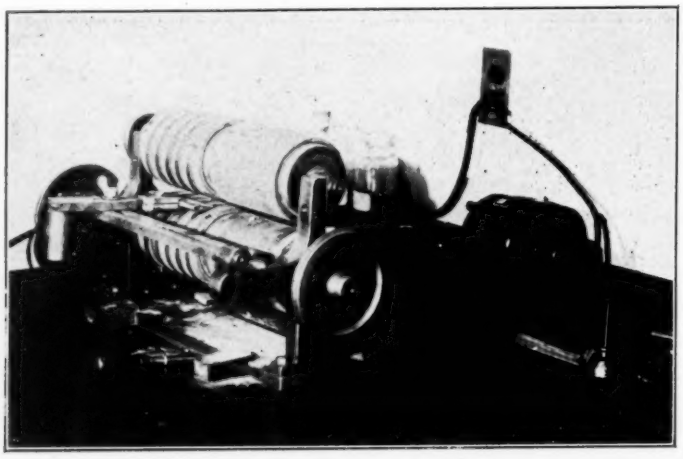

Fig. 4.—Side view of microtome knife sharpener showing the knife in position with its edge properly in contact with the finishing roller to correspond to its final bevelled edge.

The rollers are connected up by round leather belting over pulleys. The two rollers are run in opposite directions and away from the edge of the knife. The proper adjustment of the knife holder on the rocker bar is very important. The distance in or out determines the final bevel edge of the knife. Once established this should be kept the same. We found it an advantage to grind all the knives to the same bevel then to leave the knife holder locked in place and just slip the various knives in or out of place by releasing the screw on the jaw at the end which clamps the knife. The pressure of the knife on the rollers is adjusted by a spring tension on the two arms attached to the rocker bar which ride over the cam. This adjustment we have found to be very important and will probably lead to our making some further changes in design to more adequately control it. The machine in principle seems to be sound. We do feel however that many improvements can be made in the way of finer adjustments. The character of the leather strapping used is important. In the case of the spiral straps these must be kept clean and occasionally oiled. The leather rollers on the right side must be

turned down as true and smooth as possible. They are oiled and a fine grade of aloxite powder rubbed in to leave proper grinding surface.

The machine is run by a small 1/16 horse power electric motor.

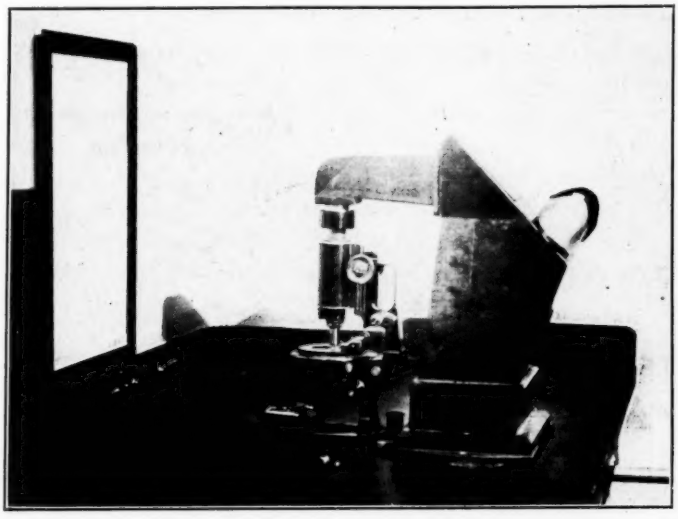

Fig. 5.—Side view of camera showing shutter box at base of microscope pushed forward to allow for focusing.

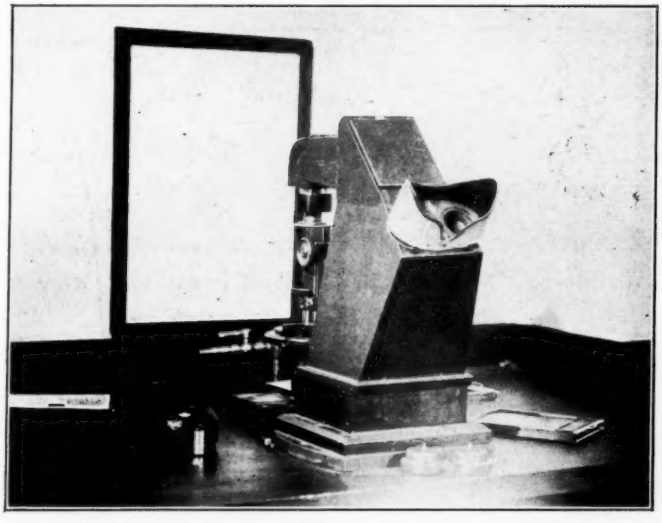

Fig. 6.—Showing the opening protected by a stereopticon shield for viewing the image while focusing and on the right at the base, a plate holder partly inserted into position for exposure.

PHOTOMICROGRAPHIC CAMERA

This was designed to meet the need for a camera which would be simple to handle and ready at hand. It is securely mounted on one's workbench with microscope and light permanently placed. Interesting or important sections can be photographed at once, no time being lost in going to another department or in making camera adjustments. Its limitations in magnifica-

tion are broad enough to allow for 90 per cent or more of the work. The lowest magnification we have found satisfactory for general purposes is x48. If lower is needed the camera can be set a little higher off the bench to allow for a greater extension of the microscope barrel. The upper limit of magnification is about x800, although we have taken pictures up to x1800. These latter are difficult to make and usually not altogether satisfactory.

The camera consists essentially of a light wooden box, light-proof, and built as shown in the illustrations (Figs. 5 and 6). A wooden spout projects forward 7 inches. It is 2¾ inches square at the camera end and reduces to 2⅛ inches square at the distal end. A piece of 1½ inch thin brass piping is inserted in a hole on the under surface of the spout at its outer end and allowed to project down ¾ inch. This provides an opening for the microscope on which should be placed a circular metal light-trap. The distal end has an opening cut at an angle of 45° which is covered by a wooden block

Fig. 7.—The camera in use, showing its ease of handling and accessibility of focusing parts.

rounded off on its upper surface and countersunk on its under surface. It is hinged to the spout. To the under surface of this block is attached a ¾ inch right angle prism set so that the rays of light coming from the microscope are deflected at a right angle through the spout to a surface mirror in the top of the camera box. The camera is constructed so that a 4×5 plate holder will slip into its base. The plate holder is held up in position by a wooden plate $4¾ \times 6½$ inches with three coiled springs under it. The upper surface is painted white. This serves as a focusing screen. It is cut down a sixteenth of an inch to give it the same level as a plate in its holder. Fig. 6 shows a plate holder on the right side of the camera partly inserted into its proper position. The camera is set on a board $7 \times 8¾ \times ¾$ inches in thickness. If a taller microscope is used one or more boards may be placed under the camera and screwed down to the table until the proper height is attained for the free movement of the microscope barrel within the limits of magnification required.

The main body of the camera is constructed with bevels to reduce its bulkiness. Its base (outside measurement) is $5\frac{3}{8} \times 6\frac{1}{4}$ inches and its height to the apex of the camera in front is 13 inches. The top is cut at an angle of 45° and toward the front an opening is left in it $3\frac{1}{2} \times 4\frac{1}{2}$ inches with countersunk edges. Over this is placed a lid with overhanging edges so as to exclude light and to the under surface of this is attached a surface mirror measuring $1\frac{1}{2} \times 2\frac{1}{2}$ inches. This mirror reflects the image from the prism down onto the screen board at the base of the camera. The length of the spout and the height of the camera is constructed so as to give a projection length from the ocular of the microscope to the plate in its holder of 500 mm. This is double the normal projection length of a microscope and therefore doubles its magnification. The image is viewed through an opening in

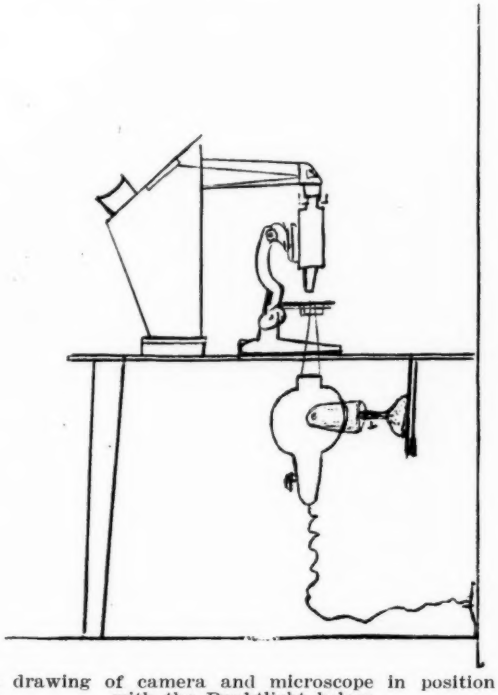

Fig. 8.—Diagrammatic drawing of camera and microscope in position on top of the bench with the Punktlicht below.

the top of the camera toward the back which is protected by an ordinary stereopticon shield as illustrated in Fig. 6. When the focus has been accurately adjusted the slide is dropped down over the viewing holes and the plate holder inserted.

As an illuminant we use a Zeiss Punktlicht. This is mounted beneath the bench (Fig. 8), on a crossbar so that it can be moved sideways as well as back and forth in order to center it beneath the substage condenser. A hole $1\frac{1}{2}$ inches in diameter is bored in the bench top to allow the light through. Once it is properly placed it will not have to be touched again. To focus it the substage condenser of the microscope should be removed. The light is then adjusted to a sharp focus on the object slide. The substage condenser is then put back into place and adjusted with its rack and pinion to

bring out the clearest delineation of the image. For very low power work we remove the front lens of the substage condenser. To control the exposure a small inverted wooden tray slides over the legs of the microscope stand and in it is bored a hole to come exactly over the beam of light. Into this hole is screwed an ordinary camera shutter of the varying speed type. This is controlled with a plunger type of release, Fig. 5. To focus the section the tray with shutter is slid aside. When the focusing is completed it is shoved back into place and the shutter used. Whatever colored screen is required is simply placed on top of the shutter as illustrated in Fig. 6.

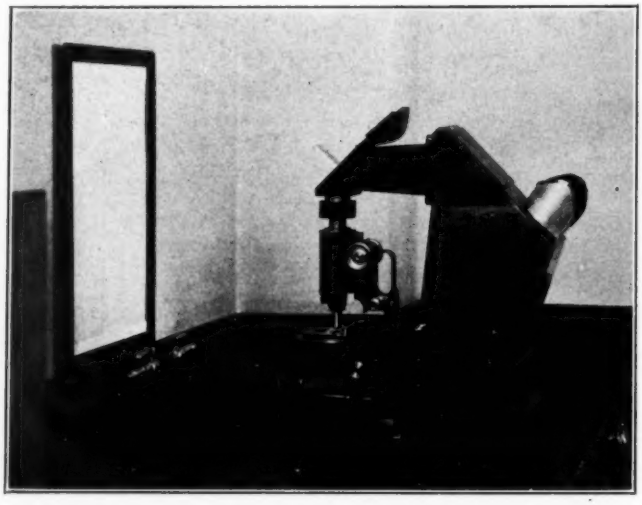

Fig. 9.—The camera being used as a projector. The image is thrown onto the screen in front by merely slipping an extra mirror into the end of the spout over the microscope.

One can see from Fig. 7, how readily accessible are all the controls on the microscope and how easy it is to focus the image and make the exposure. While the camera is constructed to use 4 × 5 inch plates we put in kits and use 3¼ × 4¼ inch plates. Lantern slides are then made by direct contact printing. If larger prints are desired enlargements may be made from the original negative.

The camera can be put to another use, namely, a projector for demonstrating slides to small groups. This is done by merely lifting the hinged wooden block on the front of the spout and inserting a small mirror into a groove cut at an angle of 45° as shown in Fig. 9. The image from the microscope is then thrown forward onto a screen placed on the wall in front of the camera.

A METHOD FOR THE STANDARDIZATION OF COLLOIDAL GOLD SOLS IN THE LANGE TEST*

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THE precipitation of colloidal gold by cerebrospinal fluid was first investigated by Carl Lange.[1] He found that colloidal gold was not precipitated by cerebrospinal fluid obtained from normal individuals, but that those from individuals suffering from certain affections of the central nervous system gave definite precipitation reactions. He observed that these reactions invariably occurred with cerebrospinal fluids obtained from patients with syphilis of the central nervous system, and considered that the colloidal gold test was of great value in the diagnosis of such conditions. These observations of Lange have been subsequently confirmed by various observers.

In 1914 Miller and Levy[2] emphasized the fact that the precipitation of colloidal gold by spinal fluids from patients with tabes differed in a fundamental manner from those obtained with spinal fluids from paretics. Miller, Brush, Hammers and Felton[3] confirmed these observations. They concluded that the reactions are characteristic with spinal fluids of general paretics, but not with those in tabes nor in cerebrospinal syphilis. They also observed that the typical paretic curve was obtained occasionally with spinal fluids obtained from syphilitic patients showing no evidence of dementia.

Mechanism of the Gold Sol Reaction With Cerebrospinal Fluid.—The mechanism of the reaction between the colloidal gold sol and the spinal fluid has been the subject of many investigations. Lange[1] assumed that the reactions were due to the presence in the spinal fluid of specific proteins. Weston[4] found that the gold precipitating substance is not the Wassermann producing substance, since separation of the two could be effected. He suggested that the active precipitating substance in the spinal fluid is a globulin, since it is precipitated from solution by ammonium sulphate, and is destroyed by heat. It is necessary to note that Weston did not consider an increase in globulin alone as indicative of syphilis because such an increase is found in the spinal fluid in many conditions other than syphilis.

Felton[5] considered that the various types of reaction were due to the antagonistic precipitating relations of albumin and globulin, the albumin exerting an inhibitory action and the globulin a precipitating action. The results obtained by Fischer[6] indicate that all four of the globulin fractions (fibrinogen plus fibrinoglobulin, euglobulin, pseudoglobulin I and pseudoglobulin II), possess a flocculating effect upon the colloid gold sol. These different globulin fractions in similar concentrations exert flocculating effects which vary in degree. Both the first and second albumin fractions fail to

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Received for publication, July 1, 1931.

cause flocculation of the colloidal gold but have a protective effect tending to prevent such flocculation. The amount of flocculation is proportional to the concentration of the globulin solution.

Spat's[7] theory that in syphilitic involvement of the central nervous system the flocculation of the gold depends upon the syphilitic antibodies was disproved by these experiments. Lange's[8] and Eicke's[9] theory that from the result of the colloidal gold reaction the strength of the Wassermann reaction may be predicted is valid only in certain cases. In some cases of cerebrospinal syphilis, the Wassermann reaction of the spinal fluid is negative in spite of considerable flocculation of the gold. The expulsion into the spinal fluid of the syphilitic regent from the infected central nervous system does not always run parallel with the exudation of the globulin.

Independently of Fischer, Cruickshank[10] made a detailed investigation into the value and mechanism of the colloidal gold test. He confirmed the conclusions of Weston that the active substance in the spinal fluid in general paretics is not dialysable and that it resides in the globulin fraction of the protein, but stated that it is not affected by heating to the coagulable point of the protein. He observed that the various types of the syphilitic reactions could be simulated by mixtures of globulin and albumin, the globulin acting as the precipitating agent and the albumin as the protective agent. He therefore concluded that the syphilitic reactions are in part due to the presence in the spinal fluid of albumin sufficient in quantity to partially obscure the precipitating effects of the globulin, and in part also to a specific alteration in the physical state of the globulin which is associated with a positive electrical charge.

It appears then that the type of reaction obtained with any given abnormal spinal fluid depends upon the balance between the albumin and the globulin content of the fluid. When globulin is present along with little albumin, the flocculation of the gold begins in the lowest dilutions of the spinal fluid and tends to disappear in regular gradations in the higher dilutions after the precipitating effect of the globulin has been dispersed by the dilution. This type of spinal fluid is rather constant in general paresis, and occurs with considerable frequency in multiple sclerosis. It gives the so-called "paretic type" colloidal gold curve in the test.

In spinal fluids containing a similar amount of globulin, but a greater amount of albumin than occurs regularly in spinal fluids of the paretic type, incomplete precipitation occurs in the lower dilutions of the fluid. The amount of precipitation increases with the dilution for a time to a peak after which further dilution brings about a regular decline in the amount of flocculation. This gives what is known as the "tabetic" type of gold sol curve. With such fluids the protective effect of the albumin must be dispersed by dilution before the precipitating property of the globulin brings about its full effect. The increasing precipitation with dilution is due to the elimination of the inhibiting effects of the albumin, while globulin concentrations remain active. Upon further dilution, the precipitating effect of the globulin is weakened so that a gradual decline of the curve occurs.

When both the albumin and the globulin contents of the spinal fluid are greatly increased over the amounts present in the above types, the same phenomena are observed as occur in the "tabetic type" of reaction. Here, however, the inhibiting effects of the albumin will be carried through a wider range of dilutions, and after the albumin effect disappears, there yet remains sufficient globulin concentrations in the higher dilutions to bring about complete precipitation of the gold. The highest dilution used routinely in the test (1:5,120) may cause complete precipitation of the gold used. If still higher dilutions are made, however, a point is reached where the precipitating effect of the globulin declines rather abruptly. This type of fluid is found usually in acute meningitis. Chronic forms of meningitis may give rise to it, but more frequently, such fluids tend to approach more nearly the reactions of the "tabetic type."

Methods Heretofore Advocated for Adjusting Gold Sols for a Standard Reaction.—One of the difficulties met with in applying the colloidal gold test has been that of standardizing the sensitiveness of the gold sol to be used from time to time in the tests. Adams and his associates[11] investigated the P_H readings of sols and found that a P_H of 5.2 yielded sols of satisfactory and fairly constant sensitiveness. In this method a mixture of 8 c.c. of the sol and 1 c.c. of a 5 per cent sodium chloride solution in triple distilled water is allowed to stand for twenty-four hours. Upon the precipitation of the gold 2.25 c.c. of the clear supernatant liquid (representing 2 c.c. of the original gold solution) is titrated with N/500 hydrochloric acid to the maximum yellow with brom-cresol-purple, i.e., P_H 5.2. According to this titration the bulk of the solution is then adjusted and the P_H reading checked.

The hydrogen-ion reading may be made on the gold sol without precipitating the gold if the dye is added directly to the gold solution and a tube of gold sol without dye used in front of the color standard as a color compensator when bromthymol-blue is used as the indicator. If the precipitation method is preferable, the whole determination may be hastened by centrifugalizing after the addition of the sodium chloride solution so as to obtain the clear liquid in a few minutes.

The older method of testing the sensitiveness of the gold sol by the use of 1 per cent sodium chloride solution is useful as a preliminary test. The addition of 0.4 c.c. of the sodium chloride to 5 c.c. of the gold sol should produce no precipitation as evidenced by a color change, while the addition of 1.7 c.c. of the 1 per cent solution of sodium chloride should produce complete precipitation of the gold. We have found that more information may be gained from this test if the amounts of sodium chloride solution added are so chosen as to bring out partial precipitation of the gold. It has been found that the above tests supplemented by the addition of a third tube in which 1 c.c. of the sodium chloride solution is mixed with 5 c.c. of the gold proves more useful as a preliminary indication of the sensitiveness of the gold. A gold sol of the proper sensitiveness will produce a reading in this tube of not greater than "2" after one hour at room temperature. The attempt to use this reaction in determining the amount of acid or alkali to be added to a gold sol for the proper adjustment of its sensitiveness has not met

with success, chiefly because the response to precipitation with this amount of sodium chloride is not delicate enough after the additions of graduated increments of very dilute acid or alkali throughout a series of tubes to bring out color changes proportionate to the amounts of acid or alkali added to the gold in the various tubes.

Considerable experience with the method of adjusting the sensitiveness of the gold sols by means of the adjustment of the hydrogen-ion reaction demonstrated to us that there was another factor aside from the reaction which influenced the response of the gold to precipitation in the spinal fluid test. In the preparation of the colloidal gold we have used the following technic: 10 c.c. of a 1 per cent gold chloride solution (Merck's Blue Label gold chloride in double distilled water) is added to 1000 c.c. of double distilled water which has just reached a temperature of 60° C. The heating is done over a Bunsen burner with the water in a 2-liter Erlenmeyer flask supported on a tripod covered with a wire gauze. Immediately after the addition of the gold chloride solution, 7 c.c. of a 2 per cent potassium carbonate solution is added and the heating continued until the temperature reaches 90° C. The flask is removed and placed on a towel folded so that several thicknesses permit handling with adequate protection from the heat. Formaldehyde, 1 per cent solution, is now added while the flask is being shaken thoroughly. Small additions are thus made until the appearance of a slight pink tinge appears throughout the liquid. Usually about 10 c.c. of the formaldehyde solution are required. The shaking is now stopped and the reaction goes on rapidly to completion. This method has yielded satisfactory clear red gold sols with a high degree of consistency. Failures occur occasionally but these can usually be traced to some variation from the standard technic, at times even to such a small consideration as the fitting of a new cork stopper to some part of the glass apparatus used in the preparation of the distilled water. Usable golds must have little or no turbidity when viewed by reflected sunlight in thick layers. The amount of this turbidity though slight varies with the different lots of gold sol prepared from time to time. The appearance of this turbidity on reflected light is an expression of the amount of relatively large particles of gold in the sol. When the amount of large particles is sufficient to give a slight violet tinge to the sol when viewed by transmitted light the sol is unfit for use.

Given two usable gold sols of like P_H concentration but differing slightly in the amount of this turbidity by reflected light it will be found that the one showing the more turbidity will be the more sensitive when used in the test with spinal fluid. This then has been found to constitute a second factor in the standardization of gold sols. It may be expressed briefly as follows: the sensitiveness of a gold sol in the reaction with spinal fluid varies directly with the size of the colloidal particles, when the P_H is constant. It follows, since acid reactions tend to increase the sensitiveness, and alkaline ones to decrease the sensitiveness, that the P_H concentrations which must be used to adjust gold sols differing slightly in turbidities to a standard sensitiveness will vary somewhat. Very clear gold sols when adjusted to this standard will show P_H readings of from 6.0 to 6.4, while the very slightly turbid ones when ad-

justed to this standard will show readings of from P_H 6.5 to 6.8. These two factors, the size of the colloidal particles and the P_H concentration of the gold sols are inseparable and must be considered of the greatest importance, for on them depends the sensitiveness of the gold. Cruickshank[10] and other investigators have pointed out that acid sols are too sensitive and alkaline ones are insensitive. Our observations have demonstrated that slightly turbid gold sols require greater amounts of alkali and higher P_H values than do very clear golds. Because of this variation of the P_H values with the size of the particles, a method of adjustment of the reaction on the strength of a test depending only on the hydrogen-ion reaction is unsatisfactory. Both factors must be considered, and can best be taken into account by the method to be presented herein.

Preserved Spinal Fluid Essential as a Standard.—In the method described in this paper, a preserved spinal fluid is essential. In the course of a large experience with the colloidal gold test, it occurred to us, that the most certain method of taking into consideration all the factors concerned in standardizing the sensitiveness of different gold sols in this test, had as its basis the discovery of a means to maintain constancy in the reacting strength of a given spinal fluid over a period of time. If this could be accomplished each new gold sol could be used in an actual test against such a spinal fluid, and by proper additions of acid or alkali different gold sols could be adjusted to give the desired reaction with this spinal fluid. This would mean that different samples could be made to react in a standard manner with this one spinal fluid. It was soon learned that gold sols adjusted to a constant reaction with a given positive spinal fluid of the "paretic type" could be depended upon to react in a constant manner with normal spinal fluids and with the different types of abnormal fluids found in the routine application of the test.

Various methods of preserving spinal fluids were tried in an attempt to find a means of maintaining a constant reacting value of a given fluid over a considerable length of time. It was found that the addition of glycerin in 50 per cent concentration offered the best possibilities in this regard. Spinal fluids showing typical "paretic type" reactions were selected from week to week over a period of a year. To each was added an equal volume of glycerin. After thorough mixing, the glycerinated specimens were stored in the refrigerator. Records were kept of the original reactions in the colloidal gold test, and from time to time the test was repeated. Of 48 specimens on which sufficient data have been recorded, the results have been as follows: all gave the

TABLE I

SHOWING MANNER IN WHICH PRESERVED SPINAL FLUIDS RESPOND IN THE COLLOIDAL GOLD TEST OVER A PERIOD

Spinal fluid I,	1/28/25....5555554321	Spinal fluid II,	3/13/25....5555432100
	3/13/25....5555554321		4/27/25....5555432100
	4/27/25....5555554321		5/12/25....5555432100
	5/12/25....3445554321		6/16/25....4555432000
Spinal fluid III,	4/27/25....5555554321	Spinal fluid IV,	4/27/25....5555543210
	5/12/25....5555554321		5/12/25....5555543210
	6/16/25....5555554321		6/16/25....5555543210
	7/17/25....3344554320		7/17/25....4455543211

original readings after four weeks; 47 after five weeks; 44 after six weeks; 38 after seven weeks; 35 after eight weeks; 31 after nine weeks; 22 after ten weeks; 8 after eleven weeks; and none after twelve weeks.

It will be noted that when deterioration begins it is evidenced by changes in the reactions with the lower dilutions of the fluids. This has been a constant finding in all of our preserved spinal fluids yielding initial reactions of the "paretic type." Partial instead of complete precipitation in the lower dilutions marks the beginning of deterioration of the preserved specimens. More important is the fact that the higher dilutions in which originally partial precipitation occurred ("4" and "3" readings), continue to give their initial readings even after the altered readings appear in the lower dilutions. These dilutions showing the initial partial precipitation are the dilutions of choice in adjusting different gold sols to a constant reaction, so that as above indicated, a preserved spinal fluid may continue to be of use for purposes of standardizing gold sols, even after it begins to show altered reactions in the lower dilutions. Their use for this purpose after the initial reading no longer appears is not recommended.

The Choice of a Spinal Fluid for Use as a Standard.—Given a method of preserving a constant reacting value of a spinal fluid over a time sufficiently long to make its use as a standard practical, an important consideration arises in regard to the choice of the spinal fluid to be used as the standard. We wish to make it clear at the start that such choice must in a sense be empirical, since a uniform reacting strength in the colloidal gold reaction is not a property of paretic spinal fluids. It is true, however, that the reacting strengths of paretic spinal fluids are more constant than that of abnormal spinal fluids from any other clinical conditions. At most the variations in the number of dilutions giving complete precipitation vary not more than two tubes on one side or the other of a hypothetical standard which yields complete precipitations in the first four dilutions (1:10, 1:20, 1:40, 1:80), and the usual variation is not more than one tube dilution over or under these dilutions. When one couples these considerations with that further consideration that the zone of sensitiveness of a gold sol for practical results in this test is confined within narrow limits, an empirical choice of the paretic spinal fluid to be used as a standard is made easier and without a great chance for variations between standards chosen by different experimental workers.

Of greater importance than the matter of the choice of a paretic spinal fluid are the properties of the sample of gold chosen as the normally reacting gold to be used in obtaining a reaction with the preserved spinal fluid. Our aim has been to make the routine colloidal gold tests as sensitive as possible by choosing a degree of sensitiveness for the gold just short of that which would give slight precipitation in the middle zone of the dilutions in the routine test with normal blood-free spinal fluids. Arrival at such a standard can be made only after extensive trial with normal spinal fluids in the regular manner of testing. Preliminary to such trials with normal spinal fluids the gold sol should be shown to have the following properties: It should be clear red and show no color change upon the addition to 5 c.c. of the gold sol of 0.4 c.c. of a 1 per cent sodium chloride solution. The addition of 1.7 c.c. of a

1 per cent sodium chloride solution to 5 c.c. of the gold sol should cause complete precipitation upon standing overnight. The addition of 1 c.c. of a 1 per cent solution of sodium chloride to 5 c.c. of the gold sol should produce upon standing overnight a color change adjudged to be midway between a "1" and a "2" reading. With spinal fluid from a known paretic, the gold sol should give a paretic type curve. The satisfactory outcome of these preliminary tests will indicate that the sample is suitable for application in the tests with a series of normal blood-free spinal fluids which will determine very definitely the desired degree of sensitiveness. Fifteen or twenty such samples of spinal fluid will suffice. Divide the sample of gold sol into three portions each of which should be of sufficient bulk to do a set of tests with the chosen number of samples in the regular manner. First, the unaltered sample is used with all of the negative spinal fluids. If upon standing overnight no middle zone precipitation occurs, the gold sol may be either of the proper sensitiveness, or it may be slightly insensitive. In this case the two remaining portions of the gold sol are used after addition to the one of 0.1 c.c. of N/5 HCl per liter; and to the other of 0.2 c.c. of N/5 HCl per liter. Routine tests with the chosen samples of negative spinal fluid are again set up with the sample of gold sol containing 0.1 c.c. per liter of N/5 HCl. Should middle zone precipitation appear, it indicates that the unaltered gold sol is of the proper degree of sensitiveness. Should none appear, the tests are made with the sample of gold sol to which 0.2 c.c. of N/5 HCl was added. The degree of sensitiveness which is represented by that just above where the first consistent middle zone precipitation appears in such tests is the one desired.

If the first series of tests with the unaltered gold sol should show middle zone precipitation, then the same procedures are employed using N/5 NaOH instead of the HCl.

The degree of sensitiveness which we employ then may be defined as that which is removed by not more than 0.1 c.c. of N/5 NaOH per liter from the sensitiveness which gives partial mid-zone precipitation with normal spinal fluids. This gives a very definite determination of the sensitiveness of the gold sol to be used in obtaining readings with the paretic spinal fluid to be used as standard, but it is obviously cumbersome and applicable only in laboratories where abundant clinical material is available for study. We have more recently been able to define the degree of sensitiveness in terms more definite by means of a standard solution of globulin (see next paper). Once this determination is made, and a reading obtained by the use of the gold sol with a paretic spinal fluid, such reading will enable one to adjust very simply subsequently prepared samples of colloidal gold to it. By the choice and preservation of positive spinal fluids from time to time the standard may be maintained indefinitely according to the plan outlined below.

NEW METHOD FOR THE ADJUSTMENT OF GOLD SOLS

Solutions required:

1. Sodium chloride solution, 0.4 per cent.
2. Hydrochloric acid, N/5 and N/1000.
3. Sodium hydroxide, N/5 and N/1000.

4. Preserved spinal fluid. This spinal fluid is preserved by the addition of an equal volume of glycerin. As shown above, spinal fluids preserved in this manner will yield constant readings over a period of several months. It is the practice in this laboratory to use no standard preserved spinal fluids more than a month old.

Technic of the Method.—The gold sol is prepared and should be allowed to stand for at least two hours before attempting to standardize its sensitiveness. The preserved spinal fluid giving initially a positive paretic type curve is then made up in the dilution at which the first partial precipitation of the gold, that is a "4" reading, is known to occur. This dilution is made in 0.4 per cent sodium chloride solution. One c.c. of the diluted spinal fluid is placed in a test tube and 5 c.c. of the gold sol is added. A "4" reading should result if the gold sol is of the proper sensitiveness. The reading is made after the tube has been allowed to stand for eighteen hours. If this reading does not correspond to the desired "4" reaction the variation may occur on either side of that desired. If the gold sol is too sensitive complete precipitation will result, i.e. a "5" reading. If the gold sol is not sensitive enough, less than a "4" reading will appear. Should the gold sol appear too sensitive, alkali must be added; if not sensitive enough acid must be used in the adjustment. The following would represent the titration scheme in case the gold sol appears insensitive:

TUBE NO.	1	2	3	4	5	6	7	8	9	10	11
N/1000 HCl	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
Gold sol	5	5	5	5	5	5	5	5	5	5	5
Sp. fld. 1 (1:320)	1	1	1	1	1	1	1	1	1	1	1
Readings	2	3	3	4	4	5	5	5	5	5	5

In this instance it is noted that the partial reaction desired (the "4" reading) occurs first when 0.15 c.c. of N/1000 HCl is added to 5 c.c. of the gold sol. From this a simple calculation serves to show that 0.15 c.c. of N/5 HCl is required per liter for the proper adjustment of the gold sol.

If the gold sol had appeared too sensitive, a similar titration substituting N/1000 NaOH for the HCl would have been used. This reading may be made after eighteen hours at room temperature.

After the main sample of the gold sol has been adjusted by adding the calculated amount of N/5 HCl or NaOH, a 5 c.c. sample is tested with 1 c.c. of the diluted spinal fluid standard. A "4" reading should result, and the gold sol is ready for use in the routine tests.

These routine tests are controlled by setting up the regular test with the preserved spinal fluid as a positive control. In using this it must be remembered that 0.4 c.c. of the glycerinated sample and 1.6 c.c. of 0.4 per cent sodium chloride solution are placed in the first tube instead of the usual 0.2 c.c. of spinal fluid and 1.8 c.c. of the saline, since the preserved spinal fluid has already been diluted with an equal part of glycerin.

Advantages of the Method and Practical Results.—This method for adjusting the reaction of gold sols is far more accurate than any heretofore suggested. The ease with which the calculations are made adds accuracy and simplicity. The time consumed in performing the test is comparatively short

for it has been our experience that the first two titrations usually suffice if the instructions for preparing the gold are followed. Consequently, the third titration becomes necessary only in special cases. The method has been in use in this laboratory during the last five years in preparing the gold sols for over 18,000 tests and has never failed to yield satisfactory results.

DISCUSSION

The absolute necessity of standard gold sols in adjudging the results of treatment in cerebrospinal syphilis is self-evident. The lack of dependable methods for the adjustment of the reactions of the gold sol and the absence of uniformity in the methods in use make it fallacious to compare colloidal gold tests performed in different laboratories. One can hardly get the correct idea of the facts clinically from such comparisons. Nor can a true idea of the results of treatment in the cerebrospinal syphilis be obtained even if all the tests performed on a particular patient are carried out in the same laboratory, because of the inability of previous tests to adjust gold sols so that they will be constantly uniform.

We believe that the method herein described will correct these conditions and will assist the clinician to adjudge properly tests performed in different laboratories, and on the same patient to show the progress of the treatment.

SUMMARY

Colloidal gold reactions of the paretic and the tabetic type may be regarded as of diagnostic value, while the meningitic reactions are less reliable.

Reactions of the cerebrospinal fluid with colloidal gold appear to be due in part to the presence of albumin sufficient in quantity to partially obscure the precipitating effect of globulin, and in part to a specific alteration in the physical state of the globulin which is associated with a positive electrical charge.

Methods of adjusting gold sols alone dependent of the P_H value of the gold are not satisfactory.

A preserved standard spinal fluid is essential.

A satisfactory method for adjusting the sensitiveness of gold sols must take into account the fact that the sensitiveness of gold sols depends on two factors; first, the size of the colloidal particles and second, the P_H value of the gold sol.

Turbid gold sols require greater amounts of alkali and higher P_H values than do clear golds.

The method herein described is accurate, simple, non-time consuming and gives uniform results. Consequently, it makes possible the proper adjudging of tests done in various laboratories and on the same patient at different times.

Addendum.—Glassware should be left overnight in cleaning solution (sulphuric acid and potassium dichromate) and then rinsed three times in tap water and three times in distilled water. Clean glassware is essential. Gold

sol contains one part gold in 10,000 parts of distilled water so that a minute trace of acid attains a high value when compared in molecular magnitude to the gold.

REFERENCES

1. Lange, C.: Ueber die Ausflockung von Goldsol durch Liquor cerebro-spinalis, Berl. klin. Wchnschr. **49**: 897, 1912.
2. Miller, S. R., and Levy, R. L.: The Colloidal Gold Reaction in Cerebrospinal Fluid, Bull. Johns Hopkins Hosp. **25**: 133, 1914.
3. Miller, S. R., Brush, N. D., Hammers, J. S., and Felton, L. D.: A Further Study of the Diagnostic Value of the Colloidal Gold Reaction Together With a Method for the Preparation of the Reagent, Bull. Johns Hopkins Hosp. **26**: 391, 1915.
4. Weston, P. G.: Does a Colloidal Gold Curve Indicate Syphilis? Am. J. Insan. **74**: 431, 1918.
5. Felton, L. D.: Cerebrospinal Fluid and the Colloidal Gold Test, New York M. J. **105**: 1170, 1917.
6. Fischer, H.: Ueber den Mechanismus der Goldsolreaktion im Liquor cerebrospinalis, Ztschr. f. d. ges. exper. Med. **14**: 60, 1921.
7. Spät, W.: Die Goldreaktion in der Cerebrospinalflüssigkeit, Ztschr. f. Immunitätsforsch. u. exper. Therap. **23**: 426, 1915.
8. Lange, C.: Die Ausflockung kolloidalen Goldes durch Cerebrospinalflüssigkeit bei luetischen Affecktionen des Zentralnervensystem, Ztschr. f. Chemotherap. **1**: 44, 1912.
9. Eieke, H.: Die Goldsolreaktion im Liquor cerebrospinalis, Münehen med. Wchnschr. **60**: 2713, 1913.
10. Cruickshank, J.: The Value and Mechanism of the Colloidal Gold Test, Brit. J. Exper. Path. **1**: 71, 1920.
11. Adams, D. K., and Scott, W. H.: The Colloidal Gold Reaction of Cerebrospinal Fluid; Standardizing and Application in Early Nervous Disease, J. Path. & Bacteriol. **25**: 142, 1922.

A METHOD OF STANDARDIZING COLLOIDAL GOLD SOLS BY UTILIZING A STANDARD SOLUTION OF GLOBULIN*

By William A. Kreidler, M.S., Ph.D., and James C. Small, M.D., Sc.D., Philadelphia, Pa.

THE method of adjusting colloidal gold sols previously described† is quite satisfactory where spinal fluids of paretics may be obtained from time to time. Since these are not readily available in laboratories of small hospitals, or in public health and biologic laboratories, we undertook the preparation of a standard solution of globulin to be used instead of such spinal fluids. A good grade of globulin available commercially was found to serve this purpose admirably;‡ and the stock solutions of it prepared as described below have been found to give identical results over periods of from five to six months. The different samples of this product purchased from time to time have been found to be very consistent in precipitating colloidal gold sols, so that the following may be recommended as a standard stock solution to be used in adjusting the sensitiveness of gold sols:

PREPARATION OF THE STANDARD STOCK SOLUTION

A 1:400 solution of edestin in 10 per cent sodium chloride is prepared by dissolving 0.25 gram of edestin in 100 c.c. of 10 per cent sodium chloride solution. After standing about two hours with occasional stirring, the solution is filtered and the volume adjusted to 100 c.c. This stock solution is preserved in the ice box (6 to 8° C.) and gives consistent readings over a period of from five to six months. In order to leave a margin of safety, the stock solution should be prepared every three months. For use, this solution is diluted 1:25 with distilled water, yielding a 1:10,000 solution of edestin in 0.4 per cent sodium chloride. It is essential to prepare the latter freshly before each titration.

METHOD OF TESTING THE SENSITIVENESS OF THE GOLD SOL

To each of a series of six test tubes containing the following amounts of 1:10,000 edestin solution: 0—0.1 c.c.—0.2 c.c.—0.3 c.c.—0.4 c.c.—0.5 c.c. is added 5 c.c. of the colloidal gold sol to be tested. After standing overnight (about eighteen hr.) readings are made as in the test with spinal fluid. If the gold sol is satisfactory the tube containing 0.3 c.c. of diluted edestin solution will yield a "4" reading and the control tube (without edestin) a "0" reading. The usual readings for the series would be:

*From the Bacteriological Laboratory of the Philadelphia General Hospital.
Received for publication, July 1, 1931.

†Kreidler, William A., and Small, James C.: A Method of the Standardization of Colloidal Gold Sols in the Lange Test 17: 259, 1931.

‡Edestin, H. P. used in this work was that of the Pfanstiehl Chemical Co., Waukegan, Illinois.

TUBE NO.	1	2	3	4	5	6
Edestin (1:10,000)	0	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.
Colloidal gold (c.c.)	5	5	5	5	5	5
Typical Reading	0	1	2	4	5	5

If tube No. 4 gives less than a "4" reading and precipitation in tubes No. 5 and No. 6 is not complete, the gold is not sufficiently sensitive and acid must be added. If tube No. 4 gives a "5" reading, the gold sol is too sensitive and alkali must be added. In order to determine the proper amount of acid (or alkali) required for adjustment a titration is set up as follows:

METHOD OF STANDARDIZING GOLD SOLS BY TITRATION WITH ACID IN THE PRESENCE OF STANDARD EDESTIN SOLUTION

TUBE NO.	1	2	3	4	5
N/1000 HCl (c.c.)	0.1	0.2	0.3	0.4	0.5
Colloidal Gold (c.c.)	5	5	5	5	5
Edestin (1:10,000) (c.c.)	0.3	0.3	0.3	0.3	0.3
Typical Reading	3	4	4	5	5

After standing eighteen hours, the reactions are observed and the tube containing the smallest amount of acid yielding a "4" reading indicates the correction to be made. For example, if the readings on the above tubes are: –3 –4 –4 –5 –5 then the adjustment necessary for 5 c.c. of gold sol is 0.2 c.c. of N/1000 HCl, or 0.2 c.c. of N/5 HCl must be added to 1000 c.c. of gold sol to adjust the reaction correctly.

If the gold sol is too sensitive, a similar titration, but using N/1000 NaOH instead of N/1000 HCl is carried out. From the amount of N/1000 acid or alkali found necessary by these titrations to correct the 5 c.c. of gold sol used, a simple calculation determines the amount of N/5 acid or alkali required for the adjustment of the bulk of the solution. The volume of N/1000 acid or alkali necessary to correct 5 c.c. of gold sol is the same as the volume of N/5 acid or alkali necessary to adjust the reaction of 1000 c.c. of gold sol correctly.

Note: In rare cases, it may be necessary to extend the titration series to more than five tubes, increasing the amounts of N/1000 acid or alkali by 0.1 c.c. in successive tubes.

It is the practice in this laboratory to set up the three titrations at the same time. If the gold is incorrect, the amount of reagent necessary for adjustment may be determined without further delay. For the past year we have been using this method of preparing the gold sols used in more than 3600 routine tests of spinal fluids. Each lot of colloidal gold was also checked with the spinal fluid method to which reference has been made.[1] Results have checked consistently; hence, we present the edestin method for adjusting gold sols as possessing all the accuracy of the spinal fluid method and emphasize the additional advantage of its being more practical in smaller laboratories where positive spinal fluids are obtained with difficulty.

CONCLUSIONS

A standard solution of edestin which precipitates colloidal gold in a manner similar to that of spinal fluids from paretics is described.

A method of employing this standard solution in adjusting the different lots of colloidal gold so that they exhibit a uniform degree of sensitiveness in the colloidal gold reaction with spinal fluids is described.

This method has been in use for more than a year during which upward of 3600 tests were made and it has been found to be accurate and practical.

THE USE OF HYDROGEN PEROXIDE IN THE MICRO KJELDAHL NITROGEN METHOD*

By Victor C. Myers, Cleveland, Ohio

In 1914 I[1] described a slight modification of the Folin-Farmer[2] micro Kjeldahl nitrogen method for urine which permitted direct nesslerization. After this method had been in use a short time, it became apparent that the procedure could be materially shortened if some oxidizing agent could be added to the sulphuric acid to hasten the final oxidation. A number of reagents were tried, but hydrogen peroxide seemed to be the best suited for the purpose. At that time (1915) Merck's perhydrol was used in somewhat diluted form, since it was found that a drop of the 30 per cent solution was considerably more than adequate for the purpose. The suggestion of the use of the hydrogen peroxide came from Dr. A. R. Rose, who was a member of the staff of the Post-Graduate biochemical laboratory at the time. The use of hydrogen peroxide in the estimation of the nonprotein nitrogen of the blood was described in this Journal in April, 1920,[2] and its use in both the nonprotein nitrogen of the blood and the total nitrogen of the urine in the first edition of *Practical Chemical Analysis of Blood* in 1921.[3] In a paper which Dr. Rose[4] published on the micro Kjeldahl method in 1925, he stated that hydrogen peroxide was used in Myers' laboratory before it was mentioned in the literature, but did not credit himself with this suggestion. Credit for the first use of hydrogen peroxide in the micro Kjeldahl method is due to Dr. Rose.

Although perchloric acid is a more active oxidizing agent, it appears to be inferior to hydrogen peroxide when the nitrogen is determined by nesslerization, apparently for the reason that there is greater formation of amines with the perchloric acid. Although amines titrate the same as ammonia they do not give the full color development of ammonia with Nessler's solution.

The reason for calling attention to the matter now is that credit is being given to Koch and McMeekin[5] for the use of hydrogen peroxide in the micro Kjeldahl method. In September, 1924 they published a paper on a new direct nesslerization micro Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia, being apparently unaware of previous publications on the subject. It is true that they advocated direct nesslerization of their blood filtrates, whereas we only described this for urine, but the hydrogen peroxide was used for the same purpose as employed by us. It might also be noted that their Nessler-Folin reagent is essentially the same and is prepared in essentially the same way as the Nessler solution (Benedict formula)† given in

*From the Department of Biochemistry, School of Medicine, Western Reserve University. Received for publication, July 2, 1931.

†This formula was supplied us by Dr. S. R. Benedict some months prior to the publication of Folin and Denis in 1916, in which the composition of Nessler's solution was discussed.

Practical Chemical Analysis of Blood in 1921,[3] except for a somewhat stronger alkalinity, necessitated by the larger amount of sulphuric acid they used.

REFERENCES

1. Myers, V. C.: Simple Methods for the Determination of Nitrogenous Constituents in Urine, The Post-Graduate **29**: 735, 1914.
2. Myers, V. C.: Chemical Changes in the Blood in Disease. I. Nonprotein and Urea Nitrogen, J. LAB. & CLIN. MED. **5**: 418, 1920.
3. Myers, V. C.: Practical Chemical Analysis of Blood, ed. 1, 1921, pp. 32, 107, 114.
4. Rose, A. R.: A Micro Method for Determining Nitrogen, J. Biol. Chem. **64**: 253, 1925.
5. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. **46**: 2066, 1924.
6. Folin, O., and Denis, W.: Nitrogen Determination by Direct Nesslerization. I. Total Nitrogen in Urine, J. Biol. Chem. **26**: 473, 1916.

THE QUANTITATIVE DETERMINATION OF CHOLESTEROL IN THE BILE*

By Robert Elman, M.D., and J. B. Taussig, M.D., St. Louis, Mo.

ALTHOUGH cholesterol determinations in the blood have been the subject of much study and have now become a more or less routine examination in many laboratories, its measurement in the bile has escaped very extensive investigation. As a result we encountered difficulties in our early attempts to find a satisfactory procedure. From the various methods described in the literature as well as from the excellent advice of Dr. Michael Somogyi we have selected a simple, economical and, we believe, accurate procedure which has been used in a series of experiments on the cholesterol function of the gall bladder.[1]

PREVIOUS WORK

Excellent discussions of previous methods can be found in the papers of McMaster[2] and Merkelbach.[3] In general two procedures have been used. The digitonin (gravimetric, Windaus) method of determining cholesterol, while perhaps the most accurate, was at once discarded as too complicated and expensive for our use. Its advantage over the simpler colorimetric (Autenrieth-Funk) method, moreover, has been investigated by many workers[3] and, for our purposes, was insufficient to recommend it. A small series of parallel determinations, moreover, between the digitonin and the present method showed close agreement, as is evident from the results described below.

The special problem relating to bile concerns that of extraction in order to obtain a chloroform solution of the cholesterol present, free from the contaminating color of the bile pigments. Elaborate procedures such as used by Doyon and Dufourt[4] were found unsuitable for routine use. In the most recent study of cholesterol in bile by McMaster,[2] large amounts of ethyl ether were used, 350 c.c. for each determination. In our early work we used this method and found we were apt to get a yellowish tinge in the extract, which we assumed was due to some dissolved bilirubin. If we dried our sample of bile on filter paper[5] and extracted with ethyl ether in a Soxhlet this yellow color was even more marked. The use of large amounts of ether, moreover, was expensive and gave no better results than the method we finally adopted. Fowweather and Collinson[6] used chloroform directly after adding sodium hydroxide to "fix" the bile pigments. We found that this still allowed too much of the bile pigment to come through which interfered with the final color reaction. Merkelbach[3] extracted an alcoholic bile mixture with ether in a separatory funnel. In our hands separation with ethyl ether was always difficult and often impossible which led to our use of petroleum ether which gave no further trouble.

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Received for publication, July 10, 1931.

METHOD

The effect of various conditions on the color reaction was first investigated in a few preliminary experiments by studying the color produced by a standard chloroform solution of cholesterol carried through the standard procedure as described below. It was compared with an inorganic standard prepared by mixing 10 per cent $CuSO_4$ and 1 per cent $K_2Cr_2O_7$ solutions so as to give an appropriate green color.[2].

For routine determinations a standard cholesterol solution was prepared by dissolving the Merck and Company preparation in chloroform so that 1 c.c. of it contained 1.5 mg. Whenever possible, sufficient bile was taken to yield about as much cholesterol as this, so that the unknown would match as closely as possible with the standard. Ordinarily 5 c.c. of bile was taken and 20 c.c. of 3 per cent KOH (in 95 per cent ethyl alcohol) added and heated on a water-bath for fifteen to thirty minutes in a 100 c.c. Erlenmeyer flask, cooled and extracted twice (or 3 times: the result was the same) with petroleum ether (maximum B.P. 80° C.) in a separatory funnel. This extraction was quite easy. In case separation did not occur promptly a few drops of ethyl alcohol were added which layered the two solutions at once.

The petroleum ether extract was then evaporated and the residue taken up in chloroform by repeated extractions, totaling not over 7 c.c., and gathered in a 10 c.c. volumetric flask. Pure acetic anhydride (2 c.c.) and concentrated H_2SO_4 (0.1 c.c.) were then added, shaken and made up to the mark with chloroform. A similar procedure was carried out simultaneously in another flask with 1 c.c. of the standard (made up also to about 7 c.c. with chloroform). Ordinarily only 2 or 3 biles were examined at one time with one standard. The conditions of light, time and temperature were the same for the standard as well as the unknowns and comparisons in a colorimeter were made within five to fifteen minutes or as soon as a good green had developed.

We compared the results of this method with that obtained (1) from the direct Soxhlet extraction of bile, dried on filter paper; and (2) from the digitonin method as described by Okey.[7] These latter determinations were kindly performed by D. J. Kooyman, for which grateful acknowledgment is made.

RESULTS

Early in our work we noted the influence of certain factors on the development of the green color, factors which have been mentioned by previous workers. To evaluate them accurately would have led us too far afield. A number of observations were made, however, which may be briefly described as follows:

Time.—The time factor is shown in the accompanying curves (Fig. 1). It is obvious that the maximum color develops rapidly, lasts for five to fifteen minutes, and then gradually fades off. Hence we made readings within this time. The two curves also show that the reaction is faster and more prolonged in daylight than in artificial light. The quality of the color was also different as will be mentioned below. Ordinarily in bright daylight the hue

becomes grey after thirty or forty minutes, whereas in artificial light or darkness it becomes yellowish instead, and after a few hours a pure yellow or brown.

Temperature.—We noted that the higher the temperature the more rapid the reaction but the more green the quality of the color, unless the light were intense. If one adds the reagents while the flask is immersed in ice water, the color will scarcely develop at all. If one then places it at room temperature it comes on within fifteen minutes but is almost a pure blue with little yellow. By increasing the temperature the blue changes more and more toward green. If one carries out the color reaction in the dark with heated reagents the green becomes almost yellow, even if the time interval is the same.

Light.—Daylight was found to produce a more intense color than artificial light or darkness as is shown in the experiments represented in Table I and the curves in Fig. 1. The quality of the color is more blue with daylight and more green with artificial light. Indeed, as mentioned above, by developing the

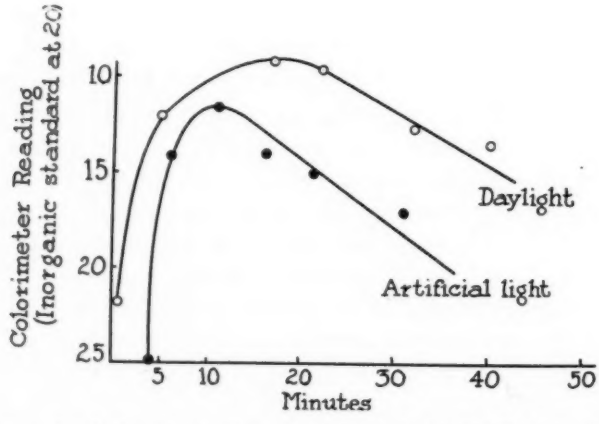

Fig. 1.—Curves of colorimeter readings of pure cholesterol following standard procedure showing (1) the changes in intensity with time and (2) that daylight causes a more intense and prolonged color than artificial light.

color with heated reagents in the dark, one can obtain an almost pure yellow color. Sunlight was found to develop a rapid blue color, which, however, loses its intensity quite rapidly, and becomes a dirty grey.

As a result of these observations we allowed the color to develop at room temperature and usually in ordinary late afternoon daylight. While being measured in the colorimeter artificial light was used but it shone equally on the unknowns as well as the standard. Reagents were added at the same rate to all flasks so as to avoid any great differences in the temperature rise, which occurs when the acetic anhydride and H_2SO_4 is added. The advantages of using a cholesterol standard, therefore, are that whatever factors vary from day to day, they affect the two solutions compared equally.

Duplicate determinations were made with many specimens and found to agree within 10 per cent and often within 5 per cent. It should be noted, however, that better checks were obtained with biles containing much cholesterol. To avoid matching the weak green elicited with samples containing little

cholesterol more bile was taken, 10 or even 15 c.c. at times. This difficulty in measuring small amounts, however, is one which applies to all methods.

In general the color obtained with the present method, even for samples containing little cholesterol, was a better one and easier to match than the

TABLE I

EFFECT OF VARIOUS KINDS AND DURATION OF LIGHT ON THE INTENSITY OF THE COLOR REACTION. INORGANIC STANDARD* SET AT 20. REAGENTS AT ROOM TEMPERATURE

EXPERIMENT	KIND OF LIGHT	DURATION (IN MINUTES)	AVERAGED READINGS
1	Moderate daylight	10	10.7
	Darkness	10	13.3
2	Moderate daylight	10	11.0
	Darkness	10	16.0
3	Moderate daylight	15	10.0
	Darkness	15	12.5
4	Artificial light	10	12.0
	Darkness	10	15.5
5	Artificial light	15	10.8
	then daylight	5	9.9
	Darkness	15	12.3
	then daylight	5	10.9
6	Direct sunlight	15	15.2
	Direct sunlight	20	21.5
	Bright daylight	15	9.9

*10 c.c. of 10 per cent $CuSO_4$ (acidified) + 0.1 to 0.5 c.c. of 1 per cent $K_2Cr_2O_7$ depending on hue desired.

color obtained from bile dried on a filter paper and extracted in a Soxhlet with ethyl ether. A series of such comparisons are represented in Table II, which shows the superiority of petroleum ether over ethyl ether, although the preliminary saponification in the former may have been responsible for the better readings. The lower values (larger readings) with petroleum ether probably indicate a greater accuracy since noncholesterol substances have not added to the color.

Table III represents comparative determinations by the present method and by the digitonin method. It will be noted that the values check very well in all but specimen 3, where the gravimetric result is higher. Since color-

TABLE II

COMPARISON OF COLORIMETER READINGS, AGAINST A KNOWN CHOLESTEROL STANDARD, OF BILES EXTRACTED WITH ETHYL ETHER (WITHOUT SAPONIFICATION) AND WITH PETROLEUM ETHER (WITH SAPONIFICATION). THE FORMER READINGS IN MOST CASES ARE SMALLER, INDICATING A STRONGER COLOR AND HENCE A LARGER VALUE THAN THE LATTER

BILE		PETROLEUM ETHER		ETHYL ETHER	
SOURCE	NUMBER	READING	MATCH	READING	MATCH
Human	1	4.2	good	3.7	good
Human	2	15.0	good	11.5	poor
Human	3	13.0	good	12.5	fair
Human	4	4.7	good	4.8	good
Human	5	1.3	good	1.5	good
Human	6	18.7	good	16.5	too yellow
Dog	1	10.20	good	7.5	too yellow
Dog	2	19.5	fair	?	too yellow to read
Dog	3	17.0	fair	?	too yellow to read

imetric results are usually higher, we went back over the steps used in this specimen and found that the filtrate before precipitation with digitonin had inadvertantly been left exposed to the air for several weeks and that the high value may therefore have been due to the presence of dust in the final precipitate.

TABLE III

COMPARISONS OF CHOLESTEROL DETERMINATIONS OF VARIOUS BILES BY THE PRESENT METHOD AND THE DIGITONIN METHOD

SOURCE	PRESENT METHOD (MG./C.C.)	DIGITONIN METHOD (MG./C.C.)
1. Human gall bladder bile	2.21	(1) 1.92 (2) 2.03
2. Human hepatic bile	(1) 0.96 (2) 1.01	1.05
3. Dog gall bladder bile	(1) 0.69 (2) 0.68	0.91 (see text)
4. Dog hepatic bile	0.083	0.087

The actual values obtained with this method are being reported for both hepatic and gall bladder biles from human beings as well as dogs.[8] Hepatic bile, collected aseptically from dogs varied between 0.04 mg./c.c. to 0.20 mg./c.c. with an average at about 0.1 mg./c.c., values agreeing with those recently reported by Enderlen, Thannhauser and Jenke,[9] who used the digitonin method, and also those by Stern.[10] They were somewhat lower than those found by McMaster.[2] Gall bladder bile from dogs contained much more cholesterol, even when as dilute as liver bile. The values varied between 0.6 mg./c.c. to 2.4 mg./c.c. in a large series of isolated determinations, averaging about 1.2 mg./c.c. In the human being, the values were still higher but here too the specimens removed from the gall bladder were much higher than those obtained from the common duct.[8] The specimens from the gall bladder agree rather well with the values reported by Fowweather and Collinson.[6]

COMMENT

The method described herein for the quantitative determination of cholesterol in bile has the advantage of extreme simplicity, requires but little reagents, and, from the data presented, seems to be as accurate as the results obtained with other methods.

The importance of saponification has recently been emphasized by Okey and others since noncholesterol substances are capable of producing the characteristic green color reaction but that they are not extractable after saponification. The use of an alcoholic solution enables the extraction with petroleum ether to be easily and rapidly carried out.

The yellowish discoloration mentioned by most workers was encountered only in specimens very weak in cholesterol and could thus be partly avoided by using more bile for extraction. It was otherwise apt to occur only when the temperature was high and when the light was poor, as discussed in some detail above. With suitable precautions, therefore, it could usually be avoided.

SUMMARY

A simple and economical method for the quantitative determination of cholesterol in dog and human bile is presented which seems as accurate as, and in many ways more satisfactory than, previous methods. Observations are also reported on the influence of temperature, light, and time on the development of the color reaction used in the method.

REFERENCES

1. Elman, R., and Taussig, J. B.: Increase in Cholesterol Content of Gallbladder Bile Following Ligature of Cyst Duct, Proc. Soc. Exper. Biol. & Med. **28**: 1066, 1931. Ibid.: Addition of Cholesterol to Hepatic Bile Subjected to Gallbladder Influence, Proc. Soc. Exper. Biol. & Med. **28**: 1068, 1931.
2. McMaster, P. D.: Studies on Total Bile; Influence of Diet Upon Output of Cholesterol in Bile, J. Exper. Med. **40**: 25, 1924.
3. Merkelbach, O.: Die Löslichkeit des Cholesterins in der Galle, Schweiz. med. Wchnschr. **59**: 620, 1929.
4. Doyon, M., and Dufourt, M.: Contribution à l'étude de la sécrétion biliare; elimination de la cholestérine par la bile, Arch. de physiol. norm. et path. **8**: 655, 1896, 5.S.
5. McMaster, P. D.: Personal communication.
6. Fowweather, F. S., and Collinson, G. A.: Certain Chemical Changes Associated With Gall Stones, With Special Reference to Relation Between Gall Stones and Hypercholesterolaemia, Brit. J. Surg. **14**: 583, 1927.
7. Okey, R.: Micromethod for Estimation of Cholesterol by Oxidation of Digitonide, J. Biol. Chem. **88**: 367, 1930.
8. Elman, R., and Taussig, J. B.: J. Exper. Med. (In press.)
9. Enderlen, E., Thannhauser, S. J., and Jenke, M.: Über die Herkunft der Gallensäuren: Cholesterin Gallensäurenbilanzen beim Hund mit totaler Gallenfistel, Arch. f. exper. Path. u. Pharmakol. **130**: 292, 1928.
10. Stern, R.: Über die Klinische Bedeutung in des Cholesterins der Galle und im Blutserum: die experimentelle Beeinflussung der Cholesterin Konzentration und des P_H in der Fistelgalle, ibid. **131**: 221, 1928.

NONELASTIC BULB FOR PIPETTES*

By John W. Williams, M.D., New Orleans, La.

DIFFICULTY in manipulation of fluids by means of the rubber teat fitted to the pipette has led us to devise a nonelastic bulb to be used in place of the rubber teat. This bulb obviates the necessity of continued pressure when different amounts of fluid are being measured. It is easily cleaned and

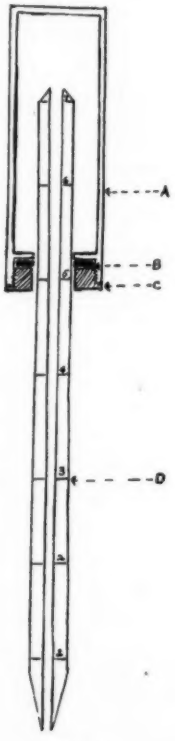

Fig. 1.

sterilized. It slides with ease on the pipette the calibrated distance desired, expelling or drawing up a measured amount of fluid. It will not easily deteriorate or corrode and is comparatively cheap to manufacture. The bulb may be made of glass or other suitable material of the desired shape, size, and capacity to fit a standard pipette.

DESCRIPTION OF CROSS-SECTION

(*A*), chamber, responsible for pressure changes at lower end of which are two flanges, one at right angles to and fitting on to the pipette; the other.

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Received for publication, July 4, 1931.

threaded and parallel to it. A piece (*C*), shaped as illustrated and fitting the pipette snugly screws into (*A*). By means of these threads, (*B*) which act as a replaceable washer, may be compressed or loosened, thus allowing variation in tightness of fit to pipette (*D*).

This device may be varied so that (*C*) is made continuous, with the flange of (*A*) and an inlay of suitable material (*B*) made in the space between (*A*) and (*C*), or the right angle flange of (*A*) made of sufficient width and tightness to fit the pipette in an air-tight manner and the necessity of (*A*) and (*B*) eliminated.

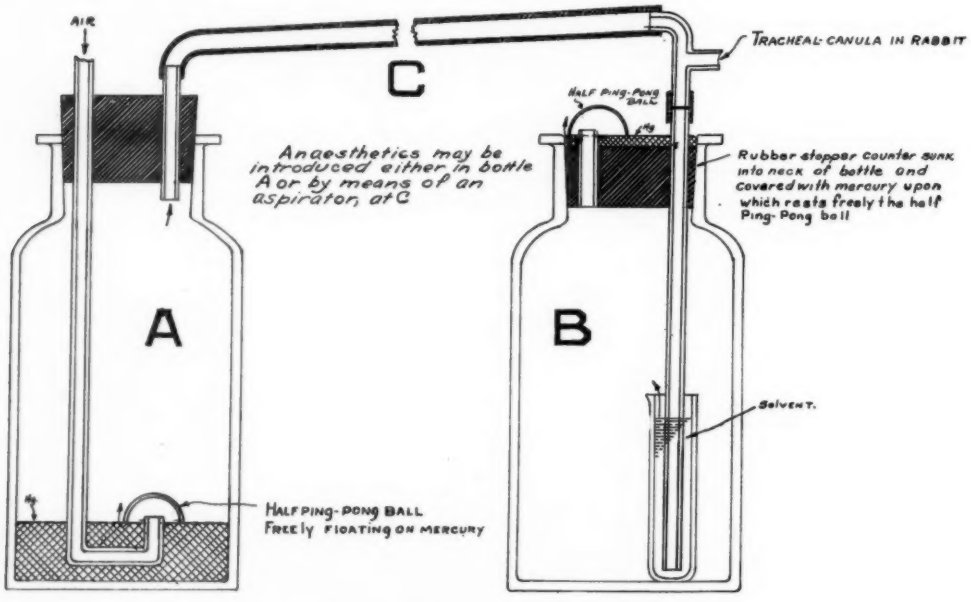
The advantage of the bulb is that it can be fitted to one pipette after another much more quickly than can the rubber teat. This will greatly speed up work in which a series of pipettes are to be used and will obviate loss of patience incident to the difficulty often experienced in the adjustment of a rubber teat to a pipette. To make the adjustment easier it is desirable that the upper end of the pipette be rounded as illustrated.

The device can be made so that a ring for the first finger may be attached to the top of (*A*). In this case the thumb and second finger will grasp the pipette and (*A*) will be moved up and down on the pipette by means of the first finger.

A SIMPLE APPARATUS FOR ABSORBING SUBSTANCES FROM THE EXPIRED AIR OF LABORATORY ANIMALS*†

By Withrow Morse, Bristol, Pa.

THIS apparatus can be made from common laboratory supplies, supplemented by a ping-pong ball obtainable at sporting goods stores. The ball is divided into its two halves at its equator and each half rested upon a mercury surface as indicated in the drawing. In order to move the column of air past these valves, it is necessary to have a force only sufficient to raise their


AIR
C
TRACHEAL CANULA IN RABBIT
HALF PING-PONG BALL
Anaesthetics may be introduced either in bottle A or by means of an aspirator at C
Rubber stopper counter sunk into neck of bottle and covered with mercury upon which rests freely the half Ping-Pong ball
A
B
SOLVENT.
HALF PING-PONG BALL FREELY FLOATING ON MERCURY

APPARATUS USED TO INSURE COMPLETE-MIXING OF EXPIRED AIR FROM EXPERIMENTAL ANIMAL WITH SOLVENT

Fig. 1.

relatively insignificant weight. In inspiration, the half-ball fits down into the mercury and prohibits all movement of air past it.

Anesthetics may be introduced directly into the containers; or, if desired, into a "U-tube" inserted into the connecting tube.

The apparatus is equally efficient in absorbing substances from the expired air of a white rat and from a large mammal such as the dog, or man himself. In the case of small mammals, the column of absorbing fluid in the

*From the laboratories of Rohm and Haas Co., Inc.
Received for publication, July 20, 1931.
†Demonstration before the Amer. Soc. Biol. Chemists, McGill University, April, 1931.

first container should not impede the passage of air; this is accomplished by keeping the column as short as possible.

For a large mammal, it may be essential to use an absorption tube (closed, expanded at the end into a bulb and holes provided in the bulb) owing to the great pressure of expiration.

The absorbing liquid, of course, depends upon the character of the substance sought. Readily volatile substances and absorbing agents should be protected by a layer of some reagent such as mineral oil, in order to reduce surface tension when applied to the receiving vessel.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEUKOCYTES: The Staff Count in Infectious Disease, Weiss, A. Arch. Int. Med. 48: 399, 1931.

A careful review of the data prepared from about 20,000 examinations of the blood performed during the past five years yields the following conclusions:

1. The leucocytosis caused by acute infections is primarily the result of stimulation of the bone marrow. This reaction of the bone marrow is a nonspecific, biologic phenomenon that depends not only on the type of organism, but on the degree of irritation caused by bacterial toxins. The neutrophilia thus brought about shows a varying percentage of immature or staff neutrophils, depending on the severity of the toxemia and the ability of the bone marrow to respond to it. A careful morphologic examination of serial blood films taken during the course of an infection will demonstrate that:

a. The peak of the staff count and the height of the infection usually coincide.

b. The peak of the staff count drops as soon as the infectious process is removed or overcome.

c. The persistence of a high staff count usually means a complication.

d. The persistence of a high staff count may mean that the infection is becoming subacute or chronic.

e. The persistence of a high staff count without the possibility of removing the infectious focus usually indicates a fatal outcome.

f. The presence of a high staff count early in the course of lobar pneumonia usually is indicative of a fatal outcome.

g. The curve of the daily staff count is more accurate as an indication of the course of the infection than the chart of the temperature.

h. The staff count is more reliable than the leucocytic or polymorphonuclear count.

2. Concomitant with the sharp drop in the staff count are a sudden increase in the number of monocytes and a return of the eosinophils into the circulation.

3. In subacute or chronic infections, one finds a persistence of an elevated staff count with an increased number of monocytes and lymphocytes.

4. Lymphopenia, which is present during the neutrophilic phase of acute infections, is replaced by lymphocytosis during the period of convalescence and healing. In subacute or chronic infections, the lymphocytes are usually increased.

For those who employ this method of morphologic examination of the blood, it is extremely important to bear in mind that a single report of a high staff count does not spell a fatal prognosis. Daily morphologic examinations of the cell are of inestimable importance, for it is on the curve that the prognosis of the case depends. It must be remembered that the changes in the blood are always to be considered conjointly with the complete clinical findings. It is also advisable to bear in mind that outstanding changes in the blood cannot be disregarded because of the lack of clinical corroboration. Nevertheless, it is also true that a definite clinical picture cannot be negated by a lack of confirmatory hematologic observations. Last, but not least, it must always be borne in mind that examination of the blood cannot always be employed as a means of diagnosis. Although one usually finds a definite biologic chain of leucocytic interreaction in infections, every now and then, owing to some unrecognizable cause, the blood picture fails to indicate the patient's condition. One must not forget that even now the reason for the appearance of the various cells in the circulation is not known, and therefore no cause can be ascribed for their failure to appear. These hematologic failures must not serve to discourage the clinician or the laboratory

worker, but should spur him on to deeper and more exact observations. Careful serial morphologic examinations in which use is made of the neutrophilic subclasses are of inestimable value in the hematologic study of all infectious diseases.

DIABETES: Study of the Five Hour Dextrose Tolerance Curve in Treated Diabetic Patients, Ralli, E. P. and Shannon, J. Am. J. Med. Sc. **182:** 395, 1931.

SUMMARY

1. The blood-sugar curve following the ingestion of 100 gm. of dextrose was studied for a five-hour period instead of the customary three hours in normal individuals and in mild, moderate and severe diabetics.

2. In the normal group the blood sugar reaches its height in from 0.5 to one hour. The blood sugar returned to a normal level at the second or third hour.

3. In the group of mild diabetics the return of the blood sugar to its starving level was at the third or fourth hour. The greatest hyperglycemia was at the first or second hour.

4. In the moderately severe group the return of the blood sugar to its starving level was delayed to the fourth or fifth hour. The greatest hyperglycemia occurred at variable periods.

5. In the severe group the blood sugar was markedly elevated for the entire five-hour period.

6. There is evidence presented that a starving blood-sugar level of over 160 mg. suggests that the patient will not return to this level within a period of five hours following the ingestion of 100 gm. of dextrose.

CONCLUSIONS

1. It is suggested that the blood-sugar level taken four hours after a meal is a better index of the severity of the diabetes than the starving blood sugar.

2. The severity of the diabetes as classified by clinical criteria was supported in all but 3 cases by the glucose tolerance test.

3. The five-hour test is found to give more valuable information in many cases than the three-hour test.

BACTERIOPHAGE: Factors in the Preparation of, Keller, M. J. Bacteriol. 22: 199, 1931.

The method following was found suitable for the preparation of bacteriophage in large amounts:

The method has two prerequisites. A bacteriophage having a lytic titer of 10^{-6} or higher, and an inoculum prepared as follows: (a) A loop of the homologous organisms is inoculated into a tube of broth (about 6 c.c.), P_H 7.6, and incubated for six hours at 37° C. (b) One part of this culture is added to a thousand parts of broth and incubated one hour at 37° C. This one-hour culture is referred to as the inoculum in the following outline.

1. To 100 c.c. of bacteriophage is added an equal amount of inoculum and the mixture is incubated for twenty-four hours at room temperature.

2. If this mixture also is clear, an equal amount of (400 c.c.) of inoculum is added and incubated again for twenty-four hours at room temperature. This "doubling" procedure is continued until the desired volume of bacteriophage is obtained.

3. At this point, approximately 100 c.c. of a six-hour broth culture per liter of mixture is added and further incubated for two days at room temperature. The mixture should now appear opalescent. If it is turbid, it should be filtered through a Berkefeld candle and the clear filtrate reinoculated with less than 100 c.c. of a six-hour culture; if it is clear, an amount larger than 100 c.c. of culture is employed for the inoculation. The aim is to add

an amount of culture that will produce an opalescent mixture within two days of incubation at room temperature. This step is based on an observation that bacteriophage filtrates remained clear if, before final filtration, they had given rise to an opalescent or a flocculent form of resistant growth which was permitted to remain in the bacteriophage for some days at room temperature.

4. Opalescence having been attained, the mixture is incubated at room temperature for five to seven days, after which period it is filtered.

5. The clear filtrate is checked for lytic potency and for possible contaminations, and if satisfactory is ready for therapeutic use.

Special Conditions. If after step 1 the mixture shows opalescence, it is incubated without filtration for about seven days to increase the titer, after which period it is filtered and the process continued with step 2. If after step 1 the mixture is turbid, indicating relatively low lytic potency, it is filtered after four days' incubation at room temperature, and an inoculum equal to half the volume of mixture, instead of the total volume, is added and incubated for twenty-four hours; if this mixture remains clear, the process is continued with step 2; if opalescent or turbid, the steps are repeated as given in this paragraph.

PERTUSSIS: Isolation and Cultivation of H. pertussis, Bailey, J. H. Am. J. Pub. Health **21**: 1144, 1931.

The following modification of the Bordet-Gengou medium is recommended.

Pared potatoes are cut into thin slices. To one kg. of potato are added 2,000 c.c. of water and 80 c.c. of c.p. neutral glycerin. This is boiled over a free flame until the potato falls to pieces, the water lost by evaporation being replaced. The material is then rubbed through a sieve and strained through a towel. The expressed juice is then adjusted to a P_H of 7. To 1 part of neutralized juice (it is rather thick) is added 3 parts of 0.6 per cent sodium chloride solution. The material is then distributed in 200 c.c. lots into 500 c.c. flasks. To each flask is added 8 to 10 gm. of agar. The flasks are then plugged and set in the ice chest overnight. If powdered agar is used it is not necessary to store overnight. The flasks are then autoclaved at 15 pound pressure for thirty minutes. When plates are to be poured the agar is melted and then cooled to about 40° C. Two hundred c.c. of sterile defibrinated horse blood, warmed to about 40°, is added and thoroughly mixed. To the resulting mixture 6 c.c. of sterile N/2 lactic acid is added and mixed. Plates are then poured and one plate is incubated for two to three days to test the sterility. This plate is not used again.

Plates of this medium may be stored in the ice chest for two weeks. It is important that the agar be not too hot at the time the blood is added, as H. pertussis grows only on unaltered hemoglobin. Indeed this fact has been used as a method for identifying H. pertussis and differentiating it from B. influenza; the latter grows on chocolate agar, the former will not until after several generations on artificial medium.

MEDIUM: for Isolation and Cultivation of Bacteria in the Filterable State, Kendall, A. D. Northwestern Univ. Bull. **23**: 8, 1931.

The "K" mediums described below were successful in the isolation and cultivation of organisms in the filterable state from various sources from which no growth could be obtained with the usual methods.

Fresh tissue from the animal or human body is thoroughly extracted with 95 per cent alcohol. Intestine has been used chiefly for this purpose. As a routine, the intestine is opened, cleaned, and ground in a meat chopper and immersed at once in about four volumes of alcohol. Extraction at 37° C. is practiced for one or preferably two days with occasional stirring. The alcohol is then removed, and fresh alcohol added. This is repeated twice, making three extractions in all. This procedure removes water, alcohol-soluble extractives

and some fat. The dry tissue residue is next re-extracted with benzol to remove most of the remaining lipoidal constituents. The benzol is removed, first by filtration, then by an air current. The extracted material is finally reground to a fine powder. It will keep indefinitely in a dry stoppered container. This dried tissue residuum is the nutritive basis for the medium about to be described. It is not necessary to limit the tissue to intestine; brain, liver, kidney, spleen and heart have been used, although in general, liver, kidney or spleen have proved to be rather less suitable than intestine. Heart muscle has been fairly satisfactory: it may be ground very fine in a meat chopper and dried rapidly in a warm air current in lieu of alcohol extraction, though alcohol extracted tissue appears on the whole to be rather better than air dried tissue. Tissue from any animal may be used. Hog intestine, however, has been distinctly more suitable than rabbit intestine, and rabbit intestine has appeared to be more favorable than dog intestine. Human intestine, which was not available when the early studies were made, is under investigation at present.

A small amount of dried intestine, two per cent by weight, or thereabouts, is placed either in a test tube or a flask of the proper capacity. Normal saline solution, or somewhat better, tyrode solution is added (tyrode solution, as used in this work, has the following composition: NaCl 8.0 gr., KCl 0.2 gr., $CaCl_2$ 0.2 gr., $MgCl_2$ 0.01 gr., Na_2HPO_4 0.05 gr., $NaHCO_3$ 0.2 gr., Glucose 0.8 gr., distilled water 1000 c.c.). Physiologically normal KCl may be used in place of physiologic saline, or distilled water may be substituted for tyrode solution. Generally speaking, however, neither KCl nor water is as satisfactory as normal saline or tyrode solution. It is best to introduce the dry tissue first, then the solution. The tissue is thereby wetted, and does not float and foam during steam sterilization. It will be noted that commercial peptone and meat extractives are rigidly excluded from K medium; this is very important. The medium thus prepared is usually slightly acid after autoclaving, hence it is necessary to add a small amount of $NaHCO_3$; usually one-half gram per liter is sufficient. The final reaction should be from P_H 7 to P_H 7.4. If the alkalinity exceeds this amount, the medium becomes distinctly more cloudy, and there is evidence of disproportionate decomposition of the nitrogenous constituents during sterilization in the autoclave. This is to be avoided for reasons that will appear below. The medium thus prepared is slightly turbid and has a tissue sediment. It is more turbid when normal saline is used as the diluent, less turbid when tyrode solution is employed.

A "clear" K medium may be prepared in the following manner: two per cent by weight of dried intestine or other tissue is thoroughly macerated, preferably with the proper amount of tyrode solution, and heated at 50° C. with frequent stirring for one hour. The solution is allowed to settle, and the supernatant, somewhat cloudy part is filtered through a sterile filter paper, which removes the greater part of the suspended substances. The filtrate in turn is passed through a sterile Berkefeld W filter, using the set-up described elsewhere (Kendall: Bacteriology, 3rd edition, figure 20, page 222). With this apparatus, there is almost no hazard from contamination during the entire process of filtration and the filtrate may be distributed in test tubes or in flasks as may be convenient. The chief advantage of the clear K medium rests in its applicability to the dark-field examination of bacteria that may be cultivated in it. Under the dark-field, the uninoculated medium is found to contain many very small, faintly greenish-yellow granules, and varying numbers of larger, bright yellow granules. The regular K mediums contain multitudes of granules of varying sizes and degrees of brightness. Bacteria in the filterable state also appear as brilliant yellow granules, but comparison with uninoculated K medium will usually afford points of differentiation.

The "clear" K medium is not recommended for isolations of bacteria or for pure culture perpetuation; there is always a possibility that bacteria in the filterable state may escape the action of heat at 50° for one hour, and remain viable in the filtrate. Many, if not most, bacteria in the filterable state will pass through a Berkefeld W filter in numbers sufficient to cause confusion later on. A reasonably satisfactory substitute for the "clear" K medium, which possesses the advantage of unequivocal sterility, may be had by filtering steam sterilized K medium, prepared as above indicated, through sterile filter paper to remove the grosser particles, then through a Berkefeld W. filter.

In spite of the prolonged extraction with alcohol, there appear to be some saccharoid substances left in the tissues which interfere very materially with the study of fermentation reactions in the K medium. This is a disadvantage that has so far proved unsurmountable. It is frequently desirable, however, to add from 3 to 5 per cent of glycerin to K medium before steam sterilization. These and other factors in the chemistry of the K medium will be discussed later. Suffice it to say here that it contains protein in colloidal solution. Some of this protein precipitates when vigorously active organisms, as the gas bacillus, are grown in it for the first transfer. It is significant that second transfers, even of the gas bacillus, in K medium usually do not precipitate the protein constituents: the organisms are changing at this time to the filterable state, a condition in which chemical activity is apparently materially modified. This K medium is an acceptable pabulum for the bacteria studied so far, which include many types ranging from those isolable from the blood of persons having influenza, common cold, rheumatic fever, and arthritis, to those microbes cultivable in ordinary laboratory mediums.

Just how much degradation the protein constituents of the K medium undergo during heat sterilization cannot be decided at this time. If, however, the mediums are carefully steam sterilized, the organisms thus far studied grow very well.

Thus far, experience has clearly shown that a single exposure to 15 pounds' live steam pressure for twenty minutes in the autoclave is ample to afford sterility, although as a matter of precaution two exposures each of fifteen minutes to live steam at 15 pounds' pressure with a twenty-four-hour incubation period intervening has been practiced in those mediums put up in flasks to receive 10 c.c. of blood for blood cultures. These flasks usually contain about 2 gm. of tissue and approximately 100 c.c. of tyrode solution. Test tubes containing but a small amount of extracted tissue, suspended in 10 c.c. of tyrode solution are sterilized but once as a matter of routine. Controls should always be carried along from the same lot of medium to afford additional information as to the sterility of the uninoculated tubes.

THE USE OF K MEDIUMS

The degree of turbidity of various lots of K medium varies somewhat, hence it is always helpful to incubate an uninoculated tube (or flask) for purposes of comparison with the inoculated one. This is usually less essential if ordinary bacteria in the nonfilterable state are being investigated, but for blood or spinal fluid cultures it is very helpful. Furthermore, in making blood or spinal fluid cultures, it is best to have the ratio of blood or spinal fluid to K medium rarely exceed 1:10; that is, one volume of blood to 10 of K medium. Anaerobic incubation at 30° C. as above indicated, usually gives the most consistent results. Cloudiness, which is the most common sign of growth, does not ordinarily become apparent before the seventh to the tenth day, unless bacteria are present in the nonfilterable state, and therefore cultivable directly upon enriched ordinary mediums. Here evidence of growth may be had often within the second to the third day, or sooner. When cloudiness is unequivocally present, transfer to fresh K medium may be made, with excellent prospects for successful subculture. It is always well to make a subculture from the original culture in ordinary, peptone-containing medium, as well as in the K medium. This is also advisable in the original culture from the patient's blood or spinal fluid. These cultures should be kept for several days, as experience has shown that two weeks or more may elapse before growths appear, though in the group of diseases discussed above, both sets of cultures in artificial mediums, even when enriched with blood, will usually be negative. Growth takes place readily, even though slowly, in K medium, however.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

*Nephritis. Its Problems and Treatment**

(For review, see editorial, page 292, this issue.)

Allergic Diseases, Their Diagnosis and Treatment†

ALTHOUGH the first edition of this work is but four years old the third edition represents almost entirely a new volume, since it has been built up to nearly twice the original size. The rapidity with which the first two editions were exhausted and the progressive increase in size of the volume are good indications of the increasing interest in the subject and the need for frequent revision and additions due to rapidly accumulating new information.

The author has added in this last edition a discussion of allergies other than inhalant allergies, particularly migraine, urticaria, and eczema. This has necessitated a change in the title from "Hay Fever and Asthma" to "Allergic Diseases." Since the allergic aspects of these various maladies are so interrelated this appears to the reviewer to be a most desirable change.

The author's contributions to allergy, particularly his pollen survey of the midwestern section of the continent, and his contributions on "allergy and intelligence" lend an authority to the book that justify the rapid appearance of three editions.

A Text-Book of Medicine‡

THE review of the first edition of this volume which appeared in 1927 stands equally well for the second edition. Osler's Textbook which stood preeminent for so many years was primarily the experience of one most unusual man supplemented by the experiences of others. Based as it was upon the great personality of the author, the question at once arose as to whether any successor could keep the volume up to date. Dr. Cecil has solved the problem in an equally satisfactory way and possibly a more permanent one in that instead of endeavoring to write an entire volume based on personal experiences, he has filled it with a large staff of contributors, each writing on that phase of medicine in which he has been especially

*Nephritis. Its Problems and Treatment. By T. Izod Bennett, M.D., (London), F.R.C.P., Physician With Charge of Out-Patients, Middlesex Hospital; Physician to the Royal National Orthopaedic Hospital; Late Beit Memorial Fellow for Medical Research, Etc. Cloth. Pages 94. Oxford University Press. American Branch, New York, 1929.

†Allergic Diseases. Their Diagnosis and Treatment. By Ray M. Balyeat, M.A., M.D., F.A.C.P. Lecturer on Allergic Diseases, University of Oklahoma Medical School; Consulting Physician St. Anthony's Hospital and to the State University Hospital; President-elect of The Association for the Study of Allergy; Director of the Balyeat Hay-Fever and Asthma Clinic, Oklahoma City. Illustrated with 87 engravings, including 4 in colors. Third Edition. Revised and Enlarged. Cloth. Pages 395. Philadelphia, F. A. Davis Company, 1930.

‡A Text-Book of Medicine. By American Authors. Edited by Russell L. Cecil, A.B., M.D., Sc.D., Assistant Professor of Clinical Medicine in Cornell University, Assistant Visiting Physician to Bellevue Hospital, New York City. And Associate Editor for Diseases of the Nervous System. Foster Kennedy, M.D., F.R.S.E., Professor of Neurology in Cornell University. Head of Neurological Department, Bellevue Hospital, New York City. Second Edition. Revised and Entirely Reset. Cloth. Pages 1,592. Philadelphia and London, W. B. Saunders Company, 1930.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

interested. This materially simplifies the problem of revision, since the individual contributors are intimately conversant at all times with advances in their own fields.

The second edition measures up to all expectations.

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*Food Allergy**

WITH the development of the allergic concept as the cause for certain diseases the method of skin testing rapidly came into prominence. The two diseases recognized as being preeminently allergic were asthma and hay fever. As the studies progressed, it became increasingly evident that the outstanding cause of these two diseases was the inhalant allergen. Some cases of vasomotor rhinitis associated with food sensitizaton were noted but these were decidedly in the minority and attention was concentrated chiefly on the inhalant allergens. Furthermore, it was observed with increasing frequency that even though a person might be definitely sensitive to some offending food the skin test was not infrequently negative. As a consequence the major interest has been given to the inhalants although to be sure ingestant allergens have not been altogether neglected.

Rowe, whose interest in the food allergens has never lagged, set himself to the task of determining if possible why skin reactions to allergenic foods are negative, and how the study of an allergic case may be successfully carried through in spite of negative reactions to foods.

This volume represents the cumulative experience of Doctor Rowe together with a very comprehensive review of the literature on food allergy. Since foods are also responsible at one time or another for all of the other clinical allergic manifestations, and since in connection with the inhalant allergies the writer has incorporated discussions of inhalant allergy, diagnosis and treatment, the volume represents a comprehensive discussion of clinical allergy in general, with emphasis on food sensitization.

For the clinician who is looking for material of practical reference value the following are to be mentioned specifically: the author's description of his own elimination diets and his discussion of the methods of dietary treatment recommended by others interested in this field: an abundance of carefully worked out recipes for individuals who must be on wheatless diets, on milk-free diets, egg-free diets and even for those who must avoid wheat, eggs and milk.

Following discussion of diagnostic methods in the study of the food allergies, the author takes up the symptomatology of food allergy including gastrointestinal allergy and the other allergies, bronchial asthma, eczema, dermatitis, angioneurotic edema, migraine, allergic toxemia, perennial hay fever, and certain other less frequent conditions.

The chapter devoted to Food Allergy in Infancy and Childhood is most important since the prevention of much suffering depends upon the early recognition of allergy. The final section is devoted to a very comprehensive summary of the literature on food allergy.

Asthma and Hay Fever in Theory and Practice†

THIS is a volume whose advent allergists have been awaiting for some time. It has been written by three men preeminent in their field, each contributing a separate and distinct section. The first section, by Dr. Coca, deals with the experimental and research phases of

*Food Allergy. Its Manifestations, Diagnosis and Treatment With a General Discussion of Bronchial Asthma. By Albert H. Rowe, M.S., M.D., Lecturer in Medicine in the University of California Medical School, San Francisco, Calif.; Chief of the Clinic for Allergic Diseases of the Alameda County Health Center, Oakland, Calif.; Consultant in Allergic and Metabolic Diseases, Highland Hospital; President of the Association for the Study of Allergy, 1927-1928. Cloth. Pages 442. Lea and Febiger. Philadelphia, 1931.

†Asthma and Hay Fever in Theory and Practice. Part I: Hypersensitiveness, Anaphylaxis, Allergy. By Arthur F. Coca, M.D., Professor of Immunology, Cornell University Medical College, Clinical Professor in Medicine-elect, New York Post-Graduate Medical School, Editor of The Journal of Immunology. Part II: Asthma. By Matthew Walzer, M.D., Instructor in Applied Immunology, Cornell University Medical College, Deputy Attending Physician Clinic of Applied Immunology New York Hospital, Chief of Allergy Clinic, Jewish Hospital of Brooklyn. Part III: Hay Fever. By August A. Thommen, M.D., Lecturer in Medicine. University and Bellevue Hospital Medical College, Director of the Allergy Clinic, Medical College Dispensary, New York University. Cloth. Pages 851. Charles C. Thomas, Publisher, Illinois and Maryland, 1931.

allergy, anaphylaxis and immunity; the second section by Dr. Walzer is on asthma, and the third by Dr. Thommen on Hay Fever. Coca's presentation of the laboratory aspects of the problem brings our understanding of the phenomena of anaphylaxis quite up to date. Into it he incorporates the many constructive observations that have come from his own laboratory but never to the exclusion of other contributions, nor does he force them into a position of more prominence than they deserve. From his position as a pioneer in immunology he has watched the subject grow and has contributed greatly to its advances.

While his section deals primarily with the experimental researches, there is a correlation throughout the discussion with the recorded clinical observations.

For the man who is looking for something primarily practical, his chapter on The Preparation of Extracts and Solutions for Use in Testing and Treatment, is altogether complete and up to date. While Dr. Coca has made several contributions on this phase of the problem in the past, in *The Journal of Immunology*, this chapter presents the most recent of his methods and should be invaluable to the allergist who is properly equipped to prepare his own extracts.

The section by Dr. Walzer on Bronchial Asthma is a complete monograph in itself. Following a very comprehensive historical survey, the author presents a critical analysis of all cases autopsied as deaths from uncomplicated asthma, reported to date. The clinical survey includes discussion of treatment. The last section of his monograph presents the most comprehensive encyclopedia at present available on allergens, and the substances into which they may be incorporated unknown to the patient.

The section on Hay Fever, likewise a monograph, by Dr. Thommen, is similarly most complete in itself. The historical survey is excellent and goes into far more detail than any of the other available reference volumes. The section on the botany of plants which cause hay fever fills a need which has been badly felt for some time. It is profusely illustrated with gross specimens and photomicrographs of pollens. The final section deals with treatment.

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*Recent Advances in the Study of Rheumatism**

THE senior author in this review of the Recent Advances Series which is being published by Blakiston is well known for his pioneer work on rheumatism. Dr. Poynton was one of the earliest investigators on the bacterial etiology of arthritis. In this volume the authors contribute a critical review of the recent investigations that have been made toward the study of the etiology of acute rheumatic fever and chronic arthritis. In their presentation they incline toward the acceptance of the evidence that bacterial infection plays a major part although they accept the equally definite evidence that metabolic derangements accompany the rheumatic state. The prevailing tendency today is to consider rheumatic fever as due to streptococcus infection and probably due to a variety of streptococci rather than to one specific organism. The weight of evidence indicates a state of allergy to the infecting streptococcus; and, once the allergic state has been developed, a group sensitization to a number of streptococci. While the most convincing evidence to this end has been produced in the study of rheumatic fever, the analogy in chronic arthritis is brought out and the possibility of allergy playing a part along with streptococcus infection in chronic arthritis is further discussed. In this latter disease, however, organisms are found much more frequently in the joints and in the blood than in acute rheumatism. The authors do not accept Crowe's staphylococcus as a cause of arthritis.

The review is altogether inclusive and the authors are to be congratulated on their ability to put as much information and as broad a discussion of this great field into as small a space as they have succeeded in doing.

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*Recent Advances in the Study of Rheumatism. By Frederic John Poynton, M.D., F.R.C.P.(Lond.), and Bernard Schlesinger, M.A. M.D.(Camb.), M.R.C.P.(Lond.) 25 Illustrations. Cloth. Pages 313. Philadelphia, P. Blakiston's Son & Co., Inc., 1931.

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., DECEMBER, 1931 No. 3

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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EDITORIAL

The Treatment of Nephritis

THE problem of therapy in nephritis depends for its answer upon the answer to the question, What are we treating? There is still much that we do not know regarding the nature of nephritis and in many respects the treatment still remains empirical. From time to time new remedies have been suggested usually based upon a new theory and urged for use chiefly in an attempt to prove the validity of the theory. Thus today we observe men urging the alkaline diuretics and others equally insistent on the value of acid salts. Curiously both procedures produce results in individual cases.

In briefly reviewing the recent concepts of therapy we will for convenience consider the subject under the following headings: Glomerular nephritis, uremia, edema, and arteriosclerotic nephritis accompanying essential hypertension.

GLOMERULAR NEPHRITIS

Of all these conditions glomerular nephritis is the only one in which we consistently observe true inflammatory reactions in the renal parenchyma. Almost invariably it is associated with bacterial infection and as a rule it is de-

pendent upon either some primary focus of infection elsewhere in the body or an antecedent acute more or less generalized infection. Glomerular nephritis may be focal in distribution or it may be diffuse. It may be acute or chronic. The outstanding urinary finding which distinguishes it from the other types of nephritis is the presence of blood and usually pus. The primary requisite in treatment is, naturally, to remove the original source of infection, in so far as possible. This involves a thorough study for infectious foci.

The prophylactic treatment is of decided importance since in every acute infectious disease we must bear in mind the possibility of glomerular nephritis as a sequel. The work of Osman and others who claim that the administration of alkalis during fever leads to an important diminution in the number of cases of subsequent nephritis is as yet unsubstantiated but is of the greatest importance if correct. Such treatment amounts to the administration of bicarbonate and citrate, during the first weeks of any severe infectious fever in sufficient dosage to render the urine barely alkaline. The same may be accomplished by selecting foods which tend to produce an alkaline urine such as milk, the citrus fruits, apples, bananas, potatoes, melons, peaches, peas and beans, cabbage, carrots, cauliflower, celery, etc., and the avoidance of acid producing foods such as wheat flour, oatmeal, rice, eggs, meat, fish, fowl and oysters. This treatment carries with it apparently no risk and appears to be of distinct prophylactic value.

Where the kidney is already involved in a glomerular nephritis, especially the diffuse type, as much rest for the organ as is possible should be obtained by limiting the food intake to the minimum necessary for the maintenance of nitrogenous equilibrium. For a maintenance diet an adult should receive not more than 50 or 60 total gm. of protein daily. Tables are now available in which the protein value of average helpings of different foods is recorded, which greatly facilitates the accurate control of the diet. In an acute glomerular nephritis as in uremia the protein intake should be still further restricted to about 25 or 30 gm. daily.

Where glomerular nephritis has developed, a slightly alkaline urine probably represents the minimum degree of renal activity and foods and alkali, should be adjusted to this end.

In focal glomerular nephritis the renal manifestations are usually so mild that they do not require special treatment. The treatment consists primarily in removing the original source of infection.

UREMIA

Uremia represents a profound disturbance of the chemistry of the body fluids as the result of the failure of the kidneys to perform their normal function. At the present time there is no conclusive evidence that uremia is associated with the presence of a foreign toxin or poison. It merely represents the accumulation of normal waste products. The proportion of accumulation of these products depends upon the normal concentrating capacity of the kidneys. Thus the kidneys normally can concentrate urea about 65 times, calcium only 1.5 times. Loss of concentrating capacity will show up in measurements of urea much sooner than in measurements of calcium. Between these two extremes

we find that phosphate is concentrated 50 times, creatinine 35 times and chloride 1.5 times.

The treatment of uremia by promoting excretion through other channels is logical. The bowels should be kept well open with saline or vegetable purgatives but not with calomel. Sweating is desirable when the response is rapid and the heart is not too badly damaged. A slow response to sweating in hot air baths is often most exhausting. Venesection is of doubtful value except for the relief of cardiovascular complications. Lumbar puncture is often decidedly helpful especially in hypertensive nephritics with impending coma. Where the spinal fluid is under decidedly increased pressure, lumbar puncture should be repeated at two or three hour intervals.

The dyspnea or, more accurately, hyperpnea of uremia is due to a true acidosis but not, as in diabetes, the result of accumulation of abnormal acid bodies. Instead it is consequent on a lowering of the alkali reserve due to accumulation of acid phosphates. Where hyperpnea exists it should be corrected by the administration of sodium bicarbonate in doses up to one teaspoonful hourly. It is worthy of note that the acidosis of uremia is often even more severe than that of diabetes. The alveolar carbon dioxide tension sometimes reaches a figure as low as nine or ten.

We do not know precisely the cause of uremic twitchings and convulsions. There is however well authenticated evidence of a diminution of calcium in the blood paralleling the increase in phosphates. The blood calcium may be as low as 6 mg. per 100 c.c.

There is evidence therefore that the twitchings of uremia are associated with calcium deficiency and therefore analogous to tetany. The administration of calcium preferably in the form of calcium gluconate intramuscularly or intravenously for the treatment of uremia is therefore logical. It may be given in one gram doses hourly for three or four doses.

Coma associated with uremia is sometimes due to an actual cerebral edema, and when this is the case it may be combated with hypertonic sodium chloride intravenously in doses of 30 or 40 c.c. of 30 per cent solution. In many cases this has produced a return to consciousness.

In treating uremia where the case has not been studied previously, it is of the greatest importance to be certain that it is of renal origin. We should always bear in mind that obstruction of the urinary tract will produce a much faster increase in blood urea and nonprotein nitrogen than will nephritis. A kidney will still maintain a normal blood nitrogen content with 75 per cent of its structure destroyed. Complete *obstruction* however will produce rapid blood changes. It is therefore of importance to rule out pathology in the prostate or urinary tract, intestinal obstruction, and congestion of the kidneys due to cardiac failure.

EDEMA

Edema may be of cardiac origin in which the treatment with digitalization and one of the coronary dilating drugs, especially the purine basis, such as metaphyllin and theocalcin, is obvious.

So-called renal edema is met with both in pure lipoid nephrosis and in

glomerular nephritis. The renal changes in nephrosis appear to be secondary to basic pathology in the tissues and capillaries. The chief trouble in the kidneys consists in cholesterin deposits in the tubules. There is no evidence of inability on the part of the kidney to excrete nitrogenous substances. A diet rich in nitrogen is therefore desirable to compensate for the abnormal loss of albumin from the blood stream. It is in pure lipoid nephrosis that the Epstein high nitrogen diet is indicated.

In addition urea in capsules in doses of from 15 to 100 grains daily promotes diuresis. Another essential feature of the Epstein treatment is the administration of thyroid extract. It should be given cautiously at first up to 5 grains daily and if no symptoms of hyperthyroidism result the dose may be increased and may at times be given in a quantity as great as 60 grains daily without untoward results. We should mention however parenthetically that there are observers who doubt that the low metabolic rate observed in nephrosis is a true observation. These believe that the increase in weight from water logging of the tissues gives a fictitiously low calculation.

According to Epstein the first indication of benefit from his program of treatment lies in a reduction of the blood cholesterol, and frequent cholesterol determinations are therefore desirable. We would emphasize that pure nephrosis is very rare, that most cases diagnosed nephrosis are actually cases of nephritis with involvement of the glomeruli as well as the tubules and that in the late stages they develop hypertension and die of uremia. The high nitrogen and thyroid extract régime described above is only appropriate in true nephrosis.

In glomerular nephritis edema is frequent and even though hypertension and nitrogen retention may not be present they usually develop later. In this case a high nitrogen diet and the administration of urea must be employed with extreme caution if at all. Other means of combating the edema are preferable. Here the salt-free diet should be applied. Food tables are readily available listing foods of low salt content and, without great trouble to the doctor or the patient, dietaries may be arranged which contain less than two grams of sodium chloride daily. Furthermore, fortunately such diets usually tend to produce an alkaline urine.

Diuretics are not indicated in the edema of glomerular nephritis. Their value lies in the treatment of cardiac edema with passive congestion of the kidneys, and of true nephrosis. Mercurial diuretics should be avoided in every form of nephritis. If the edema of glomerular nephritis does not gradually improve after removal of the original source of infection, alkalization to render the urine barely alkaline, salt poor diet, and a reduced fluid intake, then the Osman diuretic treatment with alkali may be very cautiously instituted. Osman's stock mixture consists in 15 grains each of potassium citrate, potassium bicarbonate, sodium citrate and sodium bicarbonate in equal parts of peppermint water and chloroform water to make one ounce. This mixture is approximated in the preparation called citrocarbonate. The daily dose of alkali is 180 grains of total alkali, increasing by 60 to 100 grains daily until the urine is alkaline, with a hydrogen-ion concentration of 7.0 to 7.6. This will sometimes require a total dosage of 1,000 grains daily. The increase must be continued in spite of increasing edema—alkalinity of the urine being the one criterion of

sufficient dosage. Full dosage should be maintained until edema has entirely disappeared, when the alkali may be cautiously reduced by 100 grains every three days. If tetany occurs as a result of this alkalization the dosage should be reduced by one third and calcium should be given intramuscularly every two hours for several doses.

HYPERTENSIVE NEPHRITIS

The treatment of the renal changes associated with essential hypertension, the so-called arteriosclerotic kidney or granular kidney or contracted kidney is entirely a matter of the treatment of the original hypertension—at least until the terminal stages, where we are faced with the problem of uremia. The treatment of essential hypertension scarcely falls within the present discussion. Our present understanding of this condition is that it is due to an obscure infection or intoxication in an hereditarily predisposed individual.

From this review of the treatment of nephritis it becomes apparent that the appropriate treatment for one case may be entirely inappropriate for another. This is even true where we are dealing with a single manifestation such as edema. The proper treatment therefore depends upon a very intimate understanding of the clinical pathology of the condition which we are attempting to treat.

REFERENCES

Osman, A. A.: Guy's Hosp. Rep. **78**: 386, 1927.
Bennett, T. I.: Nephritis, Its Problems and Treatment; Oxford University Press, 1929.

—*W. T. V.*

Medical News

Dr. H. Warren Crowe, D.M., B.Ch. (Oxon.), M.R.S.C., L.R.C.P. of England, will visit this country shortly for the purpose of lecturing at the Conference on Rheumatism which is to be held at Pittsburgh. Dr. Crowe is the author of *Vaccine Treatment of Chronic Rheumatic Diseases, The Treatment of Chronic Arthritis and Rheumatism,* and *Bacteriology and Surgery of Chronic Arthritis and Rheumatism* (Oxford University Press).